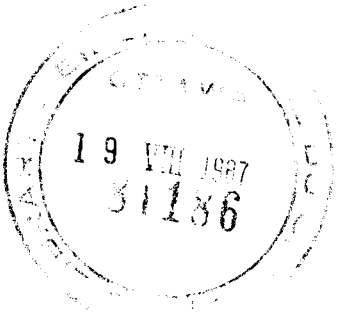
SAFETY OF NOVEL FOODS

REPORT OF A WORKING GROUP MEETING

15 JUNE 1984

PREPARED BY:
INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
P.O. 8500
OTTAWA, ONTARIO
K1G 3H9

TABLE OF CONTENTS



TABLES

APPENDICES

SAFETY OF NOVEL FOODS

REPORT OF A WORKING GROUP MEETING - 15 JUNE 1984

During the General Meeting of the International Council of Scientific Unions (ICSU) in September 1983 in Warsaw, an informal meeting of representatives of the biological unions commented upon the growing and diverse interest in novel and unusual foods: foods or food ingredients produced from materials or by processes of conversion not known to have been used to any significant extent in human diets or for continuous human consumption.

The ICSU delegates raised the question: Have methodologies been formulated adequate to determine the safety of novel foods, as above defined, for continuous consumption in significant quantities by human beings? If so, what are the methods and how widely have they been promulgated and accepted? If not, what work is in progress to devise, formulate and gain acceptance for appropriate methods, what research is necessary, and in the meantime, what cautions should be offered to scientists and technologists whose experience and interest is more devoted to biotechnological conversion and processing than to human nutrition?

In an attempt to answer these questions, a working group was convened under the sponsorship of the International Development Research Centre (IDRC) and the ICSU Commission on the Application of Science to Agriculture, Forestry and Aquaculture (CASAFA). The group met at the IDRC headquarters in Ottawa Canada on 15 June 1984. A list of participants is attached as Appendix I.

This report on the meeting consists of the following:

- Chairman's opening remarks;
- Section I: Summary of the six requested presentations;

.../2

- Section II: Summary of the discussions that followed the presentations;
- Section III: Working Group's recommendations;
- Conclusion
- Appendices.

..../3

OPENING COMMENTS

In welcoming the members of the Working Group to IDRC, the Chairman elaborated on the concerns expressed by the representatives of the ICSU biological unions.

It was noted that specific reference had been made to the processing of agricultural and biological waste materials composed of various carbohydrate, cellulosic, lignocellulosic and other organic substances. Proposed processes of conversion include aerobic and anaerobic, liquid and solid state fermentations employing a wide range of yeasts, funghi and bacteria with thermophiles and mesophiles being of particular interest since they can be grown in tropical countries without resort to temperature control by mechanical refrigeration.

Cereal straw, the by-products of woody plants and other sources of lignocellulose are of particular interest because of their abundance. The Chairman noted that workers have reported the investigation of growing collections of lignocellulytic organisms, such as wood-rot funghi, collected from tropical sources. Others have proposed thermal explosive degradation of lignocellulose followed by alternative fermentations of the derived cellulose, lignin and hemicellulose. Other researchers are screening funghi capable of progressively hydrolyzing and fermenting macro-molecular carbohydrates under tropical conditions.

Certain species of Aspergillus which showed desirable fermentation characteristics were considered a potential hazard to workers susceptible to aspergillosis and related respiratory diseases. Do other thermophiles and mesophiles present similar or other hazards?

In addition to fermentation by naturally occurring organisms the techniques of gene transfer are being proposed as a means of endowing familiar species of food yeasts with a capability to both hydrolyze and ferment cellulose and other polysaccharides.

.../4

The extensive publicity given to the biotechnologies in general and to various possible novel foods appears to be exciting expectations from and encouraging investment in related research. Is sufficient attention being given to what is necessary to ensure that whatever novel foods and animal feeds result are of a nature and composition that can be considered suitable for direct or indirect human consumption?

In addition to a variety of fermentations, microorganisms and substrates proposed for the production of novel foods and animal feeds, there is evidence of research to generate novel food plants by somatic hybridization and other techniques. Of particular interest are wide intergeneric crosses between cultivated and wild species, the primary purpose being to generate hybrids tolerant to adverse agroclimatic conditions.

The Chairman pointed out that the questions raised at the ICSU meeting and by other scientists are concerned with the availability, adequacy and applicability of methods by which to establish and monitor standards of quality in novel foods and animal feeds. The concern rests not simply with foods and feeds produced under controlled laboratory and pilot plant conditions, but with those to be manufactured on an industrial scale.

Since the principal target for many of the novel food and feed proposals appear to be the developing countries of Africa, Asia, Latin America and the Middle East, it would appear highly desirable that the standards of quality and methods of assay and monitoring lie within the institutional, material, economic and human resources available. It was suggested that the Working Group give consideration not only to the availability and reliability of chemical, physical and biological methods of determining quality and safety, but equally to the necessary resources: what institutional, material and physical facilities and equipment and, most important, what human skills are needed reliably to apply the methods proposed? What order of cost is implied to establish,

.../5

maintain and operate the necessary facilities?

What particular difficulties are likely to be encountered, particularly in producing novel foods in tropical environments? Which kinds of novel foods may present the greatest hazards to human health? In general, what words of caution should be offered to governments and potential manufacturers that are being encouraged to pursue the development and production of novel foods?

.../6

SECTION I: SUMMARY OF PRESENTATIONS

1. Presentation by Dr. S. Miller

The Centre for Food Safety and Applied Nutrition (CFSAN) of the U.S. Food and Drug Administration has been approached by some U.S. companies interested in producing novel foods, or using novel processing methods, and has made some studies of the problems these foods may present. However, the CFSAN has not come to grips with these problems in an institutional way and has no plans at this time to issue guidelines. The feeling is that for the present each novel product must be considered on its own merits. This requires some classification of novel foods.

Five categories are being considered. In order of increasing concern they are:

(a) Novel constructions or processing of traditional food materials, eg. irradiated foods;

(b) Food products that are non-traditional, but on which some experience exists, eg. foods derived from yeasts;

(c) Products constructed from non-traditional raw materials, eg. funghi; such products are of concern both as regards their toxicity and their nutritional value;

(d) Products of chemical synthesis: these are promoted for their functional properties but both their potential toxicity and nutritional value are of concern. It is now possible to produce a "bread" with no nutritional value; present regulations do not easily cover such a product.

NOTE: (c) and (d) are of equal concern.

.../7

(e) Products which are constructed from or consist of organisms resulting from genetic manipulation. There is concern not only with toxicity and nutritive value, but also with the danger of the release or escape of genetically modified organisms.

In assaying for potential toxicity chemical analysis is the most reliable first step, provided, as in the case of approved additives of known composition, precise methods of analysis exist. Proximate analyses for nitrogen, lipid, carbohydrate, fibre and mineral content are necessary but do not present a total picture. Computer assisted pattern recognition and comparison (using for example modern chromatographic techniques) may be a useful guide.

Toxicological testing of novel foods presents several major difficulties. Excess dose methods cannot be used for novel foods or major food ingredients to be consumed in sizeable quantities over long periods of time. Due attention must be given to mutagenesis, particularly with genetically engineered organisms. Animal testing studies must be multigenerational and begin at the in utero stage. Tests on novel foods must take account of human functionality and possible influences upon the immune system, behaviour and general physical performance. Before any reliable guidelines can be formulated, the probable extent and pattern of consumption over a very wide area will need to be determined.

2. Presentation by Dr. R.L. Hall

In developing suitable safety tests for novel foods due consideration must be given to adequate comparison with the familiar and traditional foods that novel foods are likely to replace or supplement. Food and Drug authorities are becoming increasingly aware of weaknesses in existing methods of testing toxicity, the difficulty of detecting subtle effects, the inapplicability of the high exposure levels customarily used with minor ingredients and food additives to obtain observable results with limited numbers of animals, the possible

occurrence of false positives and, in the case of complex food mixtures possible delayed toxic effects which may be obscured by other earlier effects.

In determining the safety of totally novel foods each food must be approached as a separate entity. While reliance may be placed on established tests, new methodologies might be introduced that are appropriate to the food being examined and its potential pattern of use. It is not possible to specify a standard test protocol to cover all possible novel foods.

Table I attempts to indicate the relevance and utility of different methods of analysis and assay for four aspects of novel foods: (1) toxicity; (2) nutritional value: (3) tolerance; (4) acceptability.

Column 1 "Toxicity" indicates the extreme difficulty of establishing safety (i.e. non-toxicity) of a novel food by animal tests. In examining novel foods due attention must be given not only to chemical composition but to the production methods by which the foods are to be manufactured.

Considerable difficulties are presented by concern for individual responses among consumers. In this regard it may be necessary to gather data relevant to intolerance and allergenic response to traditional foods among human populations in different regions.

In any event, staged and carefully monitored introductions of all novel foods is essential.

..../9

TABLE I

RELEVANCE OF TESTS FOR SAFETY OF NOVEL FOODS

ASPECT		TOXICITY	NUTRITIONAL VALUE	TOLERANCE	ACCEPTABILITY
METHOD					
PREDICTIVE	T	Not done	xx	U/I	U/I
ESTIMATED/CALCULATED	N	xx	xx	U/I	U/I
CHEMICAL ANALYSIS					
- PROXIMATE	T	xx	xx	U/I	U/I
	N	xx	xx	U/I	U/I
- IMPURITIES/ CONTAMINANTS	T	xx	U/I	U/I	U/I
	N	xx	U/I	U/I	U/I
- DETAILED/ COMPARATIVE	T	Rare	U/I	U/I	xx
	N	xx	U/I	U/I	xx
METABOLIC/ PHARMACOLOGICAL	T	largely known	x - largely known	x	U/I
	N	xx	xx	x	U/I
SHORT TERM TESTS	T	xx? rate	U/I	U/I	U/I
	N	xx?	U/I	U/I	U/I
ANIMAL FEEDING TESTS					
- ACUTE	T	Imposs.	U/I	U/I	U/I
	N	Imposs.	U/I	U/I	U/I
- SUB-CHRONIC	T	Not done	largely known	U/I	U/I
	N	xx? insensitive	xx	U/I	U/I
- CHRONIC	T	Not done	largely known	U/I	U/I
	N	x? insensitive	x?	U/I	U/I
- HUMAN TRIALS	T	largely known	largely known	xx	xx
	N	x, Ultimate criterion	x, ultimate criterion	xx	xx

T = Traditional; N = Novel; xx = customary and useful; x = occasionally done but value not well established; U/I = Useless/Irrelevant

SOURCE: Dr. R.L. Hall, President IUFoST and Vice President Science and Technology, McCormick & Company Inc.

3. Presentation by Dr. Narasinga Rao

The ability of many of the less developed countries to implement testing and control of non-traditional foods is limited. Dr. Rao reviewed India's resources in this respect and discussed how India's efforts to develop new or little used foods were being adapted to the resources available.

India has emphasized the evaluation of grains and legumes that are little known outside India but have a history of local or tribal usage. Examination of the nutritional status of selected communities will provide evidence on the potential of such crops for wider use.

They are also trying to increase the yields and production of conventional oilseed crops and to evaluate minor oilseed crops. India suffers a severe shortage of dietary fats and oils: imports are necessary to maintain an average per capita intake of 13 g/day. Tree seeds are being tested, as also are the oils extracted from agro-industrial by-products including rice bran, rubber seed and tea seed. Toxicological tests have been done on various oilseeds; chemical tests for cyclic and epoxy fatty acids are being done.

Some interest exists in producing single cell protein from petroleum substrates. The principal nutritional interest in India however is in increasing the availability of calories rather than in protein supplements and there is not a great interest in SCP as a major ingredient of diet.

Gaining acceptability of novel and unfamiliar foods is inevitably a source of major difficulty.

A five year program has started to develop microorganisms high in digestible lipids grown on substrates composed of readily available and unused biomass. Chemical analysis to determine the fatty acid composition of the lipids is generally considered adequate in determining wholesomeness.

.../11

Some macro-algae have been examined as potential food sources but in general the acceptability together with high ash and fibre content present difficulties.

4. Presentation by Dr. S. Gunner

Dr. Gunner expressed agreement with most of what Dr. Miller had said. Like the U.S., Canada is not attempting to develop specific guidelines for novel foods, but is trying to take a pragmatic approach. Where applicable, the controls designed for food additives can be adapted to novel foods. The Health Protection Branch of Health and Welfare Canada grades novel foods into three categories:

(1) Those of known history; very little needed in the way of safety requirements, eg. pea protein extracts and isolates;

(2) Intermediates, eg. concentrated protein products from rapeseed or soybean;

(3) Radically new products, eg. SCP or genetically modified products.

Health and Welfare Canada tries to avoid an overly rigid approach to food evaluation preferring to judge each new or modified food upon its merits, a philosophy which has worked satisfactorily in formulating and implementing Canada's food additives legislation.

Future action will probably involve:

(1) Development of a hierarchy of needs by means of which the degree of evaluation and testing required for a novel food can be decided on a case by case basis, perhaps from a "decision tree";
(2) Refining existing tests for application to novel foods;
(3) Emphasis on factors that will arise as a novel food is introduced into common usage. A need is foreseen for close monitoring of consumption, and for gathering data on reports of allergies and intolerances. Animal feeding tests will not detect these adverse

.../12

reactions; new testing methods are needed, possibly including cultures of human tissue.

5. Presentation by Dr. N. Scrimshaw

Dr. Scrimshaw gave a brief history of the Protein Advisory Group's (PAG) Guidelines 6, 7, 12 and 15 (Appendices 2, 3, 4 and 5) and outlined the changes made during the recent review of these guidelines by the United Nations University (UNU). Points of major concern and a number of examples garnered from Dr. Scrimshaw's experience were outlined.

Many traditional foods induce allergenic or intolerance responses in some individuals. Animal feeding tests are of no value for detecting or quantifying these reactions. What frequency of such complaints should be tolerated in test human subjects or in the general consuming public is far from being decided.

It is generally recognized that toxicological studies in experimental animals and practical feeding studies in domestic animals are satisfactory for determining the suitability of novel protein sources for animal feeding, but do not assure their acceptability and tolerance for human feeding. It is sometimes suggested that the products from animals fed novel protein sources should be submitted to biological testing, but this is impractical. The best assurance comes from testing of the material before feeding. However, any substances detected in abnormal quantities in these products can be submitted to biological in vivo or in vitro evaluation. For this reason there should be an initial identification of any abnormalities in physiological state or composition of the carcass and organs of animals fed unusual feeds.

Before embarking upon tests of novel foods by human subjects, a comprehensive and detailed evaluation needs to be made of the raw materials, processes of production and conversion, chemical analyses including both macro and micro components, and results of feeding tests over a range of laboratory animals.

.../13

The incorporation of cottonseed flour containing a low level of gossypol into the vegetable mixture INCAPARINA in Guatemala gave rise to no detectable effects in animal feeding studies, that included pathological examination of testes and other tissues, or when fed to human subjects. Years later it was demonstrated in China that gossypol inhibits spermatogenesis at similar levels. Presumably, the cooking to which the INCAPARINA is submitted sufficiently inactivated the gossypol, but clearly the animal testing was a wise precaution and would have detected spermatogenic effects.

The Clinical Research Center at MIT has conducted extensive tests of yeast and bacteria products grown on a variety of substrates ranging from molasses and sulfite to alcohol and normal alkanes. Although all of these had been extensively studied in acute and chronic tests with experimental animals, most of them caused some gastrointestinal or allergic response as originally fed. It was usually possible to find a processing procedure that inactivated the substance responsible.

For example, an ordinary C. utilis grown on beet molasses and used routinely in processed foods in Europe caused a high prevalence of skin allergies when heat shocked at 80 degrees C. to reduce its DNA content. This effect was completely eliminated by raising the temperature of the treatment to 140 degrees C. for an even shorter period. Both products were completely free of detectable adverse effects when fed to rats. These results further illustrate the influence of and need for careful monitoring of processing conditions.

Yeasts grown on normal alkanes have a higher content of C-14 to C-18 hydrocarbons than most foods. However, they are no different from those in the mineral oil used in much higher concentrations without restriction to polish fruits, coffee beans etc. and have no adverse pathological implications at the levels involved. More attention has been paid to the presence of odd-numbered carbon chain fatty acids in micro-organisms at higher concentrations than in most common foods and their similar

.../14

presence in the fat of animals fed alkane grown yeasts. However, exhaustive studies that have involved not only feeding but also investigations at the ribosomal level, have shown that there is nothing abnormal about the metabolism of these fatty acids. Moreover, they are normally present in some common foods. Despite this, and then acceptance for animal feeding in other European Countries, activist groups in Italy have successfully blocked approval of these yeasts.

The revised PAG/UNU guidelines should now be widely circulated and examined critically so that they can be kept up-to-date through periodic revisions. A joint task force of the relevant International Scientific Unions carried out the recent revision and similar task forces should be appointed for this purpose in the future.

6. Presentation by Dr. J.W. Bridges

Dr. Bridges outlined the organization of the UK Advisory Committee on Novel Foods, which is advisory to both the Ministry of Health and the Ministry of Agriculture, Fisheries and Foods, and discussed its working procedures and philosophy. The Committee tries to work with industry and avoid adversarial situations. Companies are not legally required to submit data to the Committee but industry itself tends to favour full submissions. The Committee has now completed a draft set of test guidelines for novel foods (Appendix 6). The UK Committee considers that novel foods should be considered quite separately and independently from food additives in formulating legislation and safety for guidelines. On the other hand, the Federal Republic of Germany proposes to modify existing food additives safety guidelines for application to novel foods.

Some particularly interesting aspects of the UK guidelines include:

(1) They accept use of only two test levels in animal feeding trials;
(2) Acute toxicity data are not required;
(3) Metabolic studies are not emphasized except for known minor components;

(4) Broad comprehensive testing protocols are required each specifically designed for the novel food under consideration.

In response to a question, Dr. Bridges indicated that his Committee is encouraging close cooperation with medical authorities and general medical practitioners who work in the regions where novel foods are being test marketed.

NOTE: A copy of the British Memorandum is attached as Appendix 6

SECTION II: SUMMARY OF DISCUSSIONS

The following is an attempt to summarize the principal points of discussion and recommendation.

Public Attitudes Towards Food Safety

Several participants noted that concerns expressed by the general public or promoted by public interest groups may inhibit the manufacture and test marketing of novel foods even in cases where scientific evidence suggests that adequate safety precautions have been observed. Examples cited included: (1) objections to lipids, synthesized by microorganisms, that contain an odd number of carbon atoms, in spite of the absence of any laboratory evidence of toxicity or unwholesomeness; (2) the government of an African country discouraged the production of triticale on the grounds that it contained "toxic" alkyl resorcinols in spite of (i) analytical data which showed the levels to be lower than what is present in the rye grain and (ii) animal feeding tests carried out in Canada in which animals received five to ten times the intake of alkyl resorcinols present in rye flour without adverse effects.

Allocation of Costs of Testing for Safety

Though it is unlikely that small-scale manufacturers could afford the very high cost of the tests required to demonstrate safety in novel foods, it was generally agreed that in most instances the cost of demonstrating safety must be borne by the company proposing to manufacture and market. It was pointed out that in most developing countries relatively few manufacturers could afford the total cost of following such safety guidelines as are recommended by the UK Committee. Where it may be in the national interest to consider the introduction of novel foods, much of the cost of determining and continuously monitoring safety during production, distribution and consumption would have to be underwritten by government agencies. In general, however, the Working

.../17

Group believed that the highest priority should be given to increasing production of traditional foods and the processing of familiar well accepted raw materials. Unfamiliar proteins are particularly liable to lead to undesirable reactions.

The risk and high cost of litigation in the U.S. and other industrialized countries was discussed at some length. Consumers groups are well organized though not always as well informed as they might be. Food science organizations in many countries are giving more attention to public education and information concerning the composition and quality of both traditional and comparatively novel foods. Nevertheless, many prejudices and misconceptions are still evident which, together with recent court decisions in the United States that have emphasized company liability, may seriously constrain future attempts to introduce novel foods.

Tests and Testing Procedures

A number of participants recommended an increased emphasis on chemical analysis of novel foods. Experience with irradiated foods has shown that detailed chemical analyses were helpful. Dr. Miller stated that the CFSAN laboratories were exploring the possibility of developing analytical patterns for traditional foods which might ultimately be used for comparison with those of novel foods; unusual components in the patterns might then be selected for further study. While analytical techniques may help define nutritional quality and some toxic agents possibly present in novel foods it was noted that they are unlikely to substitute for toxicological testing in view of the possible presence of chemicals whose toxicity has yet to be recognized.

The reasons for the introduction of novel foods may be significantly different in various countries of the world. For example, in the U.S. and other industrialized countries where people would like to eat as much as they wish without gaining body weight, research has been devoted to

.../18

the production of products which closely resemble in appearance and flavour traditional foods but which contain reduced or no nutritive value. The use of synthetic low calorie sweetening agents in soft drinks and confections is widely familiar. A new generation of novel foods may provide substitutes which reproduce the technological and functional properties of carbohydrates, lipids and proteins but which are devoid of any nutritive value. These must be considered quite distinctly from proteins and lipids synthesized by microorganisms from various biomass substrates. Conventional tests by laboratory animals of nutritive value and toxicity cannot be considered since there will be no growth response from a material which contains no nutritive value.

Dr. Scrimshaw reported that the most common adverse effects noted in human testing of novel foods were gastro-intestinal disturbances and cutaneous rashes. In yeast and bacteria derived products most of the gastro-intestinal problems related to the nucleic acid content can be overcome by various procedures to reduce the content. However, these seem frequently to release relatively low molecular weight proteins that cause allergic responses, and require further processing to remove. Thirty day feeding studies have been sufficient to detect these problems and longer tests have had no advantage. One problem is that the processing to remove allergic substances should be closely monitored and there is no satisfactory way beyond process control to do so. When individuals with leukocytes sensitized to the antigen are available, a lymphocyte stimulation test can be used, but this is complex and costly. Moreover, the sensitivity wears off a few months after exposure.

The need for information on the frequency with which intolerance and allergenic reactions occur with traditional foods was noted by several participants. Dr. Miller reported in some studies that 5% to 60% of sampled populations reported intolerances to various foods. Severe intolerance reactions to a novel food justifies its immediate rejection, but rejection because of mild reactions in a few test subjects is hard to justify without baseline data gained from studies of traditional foods.

.../19

Two participants noted the need for studying the response of the gastro-intestinal tract during human testing of novel foods. The long resistance times in humans may permit development of adaptive bacteria and production of foreign substances. These would not be detected in rat feeding trials because of the shorter residence time in the gut.

The inappropriateness of a safety factor approach for a novel food intended for use as a major human food was mentioned. The inapplicability of the excessive dose approach to toxicity testing of such products in laboratory animals was also recognized. Dr. Scrimshaw emphasized that in human testing dual control groups are recommended especially if excessive diarrhea is evident.

The minimum number of humans that must be exposed during a preliminary feeding test was discussed. Dr. Holub noted that a test on 25 people may not detect a 5-fold elevation in toxicity. Dr. Scrimshaw replied that the fewest possible number should be exposed in a preliminary trial. He recommended a compromise of 25 to 30 persons in a double blind, cross-over test. Thus only 12 to 15 humans would be exposed in the first sequence. To date, preliminary experiments of this size have served to send all projects "back to the drawing board".

Close Monitoring at All Times

Several participants emphasized the need for close monitoring of any novel food approved for use, including monitoring of production, processing, marketing and exposure (consumption). Points raised in this regard included:

(1) The necessity of assuring that the novel food being marketed is identical in all respects to the material produced and tested in the laboratory; quality control procedures for novel foods will need to be more stringent than is required for traditional foods with which a long experience has been gained.

.../20

(2) Monitoring should give special attention to micro-flora, particularly in fermented foods since undesirable organisms can grow together with the organisms designed and intended to bring about the fermentation desired.

(3) Distribution and human consumption of novel foods should be phased in gradually and any adverse affects noted and reported by competent medical authorities. It was emphasized that controlled exposure to a major novel food will be more difficult to control than the consumption of permitted food additives.

(4) Particular caution must be exercised in distributing novel foods to such vulnerable groups as pregnant and nursing women, young children, people who are sick, the malnourished and those who have a history of specific intolerances and allergies.

Potential Risk to Workers

The potential risk to workers in factories producing novel foods was discussed. Many live organisms are more dangerous when inhaled than when ingested, a fact illustrated by experience with what appeared at first as a particularly suitable Aspergillus. This organism both hydrolyzed and fermented root starches, being most active at close to pH 3.5, converting inorganic nitrogen to a crude protein (dry weight) content of ca. 35%. Unfortunately, the Aspergillus formed aerosols which could be extremely hazardous if inspired by persons susceptible to aspergillosis.

It was also noted that some flavour ingredients and cereal grain flours may be allergenic in contact with skin and respiratory tissue though harmless when ingested.

Possible Adverse Effects of Rigid Protocols

The possible hindrance that could be caused by strict, inflexible controls on the most exemplary of novel foods was of concern to some participants. Excessive testing could put unnecessary demand on the economic, institutional and human resources of a developing country. Rigid protocols can cause long delays in the introduction of novel foods.

Corroborative to the above, a concensus developed that all guidelines for the safety of novel foods should be as flexible as possible. Tests should be required on a pragmatic, case by case basis (Dr. Gunner's "decision tree" idea). In evaluating the safety and suitability of novel foods, regulatory agencies will need to consider the whole body of evidence and not attempt to rely upon any specific or narrowly defined test.

A GRAS List for Foods

A suggestion was made that it might be possible and desirable to develop a GRAS (generally recognized as safe) list of novel food constituents, and that such a list might allow some testing to be bypassed for some novel products. Discussion of this point centered on the activity of JECFA (Joint Expert Commission on Food Additives), an FAO/WHO body, and of the Codex Committee on vegetable proteins. Dr. Hall pointed out that the U.S. GRAS list is defined as "safe under conditions of intended use", the intended use being clearly specified. Furthermore, the GRAS list was developed mainly to cover food additives and micro-ingredients rather than novel foods to be consumed in relatively large quantities. While recognizing the value of the GRAS concept in the U.S. context in which it is applied, the working group was not able to resolve how such a concept might be applied to novel foods developed for wide-scale consumption.

Draft Guidelines

The draft guidelines presented by Drs. Scrimshaw and Bridges (Appendices 2 through 6) were compared and discussed in some detail. It was emphasized that these guidelines should not be interpreted as advocating the use of novel foods. One participant suggested that the guidelines should differentiate between "essential" and "desirable" tests but a case-by-case approach seemed to be preferred. Dr. Scrimshaw noted the need for international guidelines even though various countries may prefer to develop their own. In answer to a question, Dr. Scrimshaw indicated that the UNU was willing to continue to take responsibility for

.../22

periodic revisions of its guidelines for novel foods and asked whether an attempt should be made to consolidate them with the new British guidelines being developed. Participants agreed that harmonization would be desirable but that they did not need to be identical. The present revised UNU guidelines need the addition of suggestions for manufacturing and handling and more emphasis on the gradual introduction and monitoring of the novel product.

Need for Continued Research

The need for more research was repeatedly mentioned. Dr. Miller remarked that "an incredible amount of research is needed, much of it very difficult and complex". He asked how can the effect of a novel food on human functionality including the immune function, and upon human behaviour be determined. The research needed will call for a very high standard of scientific capability combined with a practical working knowledge of the nature, process of manufacture and probable pattern of distribution and consumption of all novel foods under consideration. Several specific proposals for future research were offered (see Section III.3).

Human Resources

Though in many countries the physical, laboratory and material resources are inadequate to the task of developing and continuously monitoring the safety of novel foods, most evident throughout the world is the need for increased, suitably trained human resources. It was noted that in Canada the number of the PhDs in agricultural sciences retiring this year from the Canadian government and universities will be roughly double the number of new doctoral graduates. Throughout the world the proportion of graduates in social sciences, humanities and the arts is increasing in relation to graduates in natural, physical, health and agricultural sciences. There is evidence to suggest that national research institutions often display greater enthusiasm for the development of new technologies than for the continued monitoring of safety and desirable quality. Dr. Miller expressed the opinion that the

scientific disciplines taught in universities to food science graduates are not adequate to cope with the immense complexity of the novel foods and manufacturing processes foreseen in the future. Of particular need are graduates competent in both toxicology and in nutritional biochemistry. Developing countries in particular need to establish reliable food regulatory agencies before embarking upon extensive research and development programs in pursuit of novel foods.

.../24

SECTION III: RECOMMENDATIONS

1. Recommendation Concerning the Consolidation of Guidelines

The working party recommended that the UNU continue its efforts to produce international guidelines for the testing of novel foods. As a first step it is suggested that the present PAG/UNU guidelines be revised and consolidated, taking into consideration the UK draft document and the discussions of this working party. The revised document should, to the greatest extent possible, include guidelines for the conditions of manufacture. Guidance for the cautious introduction of any approved novel food might also be included, bearing in mind the social and dietary factors that may influence food acceptability and tolerance.

It was also recommended that the British Committee on novel foods take account of the concerns expressed by the working group during the further revision of its guidelines.

Dr. Hall, President of the International Union of Food Science and Technology (IUFoST) and Dr. Scrimshaw, a past President of the International Union of Nutrition Sciences (IUNS) agreed to establish joint working groups to consider specific aspects of the subject of the safety of novel foods, in particular how best to apply the "decision tree" approach.

Given the importance of reliable methods of chemical analysis it was suggested that the International Union of Pure and Applied Chemistry also be invited to cooperate with IUFoST and IUNS.

2. Recommendations to National Governments

Because of the complexity of the subject, the many uncertainties by which it is constrained, the extensive facilities required and the high cost of determining and maintaining safety in uniquely novel foods, all governments are recommended to approach novel food development with considerable caution.

.../25

Every government must individually weigh the risks versus the benefits inherent in the introduction of a novel product. A major factor to be considered in making this decision is the availability of resources essential for the task: information/data, research, technical, human and institutional. Only when such resources are adequately available, and backed by an effective system for technological and scientific training, can the full benefits be gained from the successful introduction of a novel food. Appendix 8 gives a summary of the approximate costs of testing various categories of novel foods, as well as the human and financial resources necessary to establish a minimum testing laboratory.

Establishment of the necessary infrastructure to assure the safety of all food produced or marketed is an essential step towards the WHO goal of full health for all persons. Health for all requires adequate food for all, and the assurance of safe food supplies requires the appropriate infrastructure. Because of high costs of research, limited resources, and the large amount of research that needs to be done, unproductive duplication of efforts should be minimized by encouraging effective international collaboration.

3. Recommendations Regarding Research

The following is a summary of research priorities suggested during the course of the meeting as well as research proposals subsequently submitted by the members of the Working Group:

(A) Development of more sensitive/appropriate model systems.

Present routine testing methods are, for various reasons, not effective in detecting a number of likely adverse effects of novel foods in man such as:

- behavioural changes, e.g. irritability
- immunological modifications, e.g. allergy
- skeleto-muscular alterations, e.g. athritis
- gastrointestinal intolerance, e.g. associated with gut microfloral changes.

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Priority should be given to developing systems/detection methods for these lesions. It may well be that the rodent strains presently used in other aspects of toxicology are not appropriate to detect the above effects and that other species should also be examined. However, in the case of gut microfloral changes it may be possible to develop a more appropriate model by seeding gnotobiotic animals with a microflora "cocktail" which resembles that of man.

The test systems also need to be improved for the assessment of the mutagenicity/genotoxicity of novel foods, as well as the immune response to foods both in vivo and in vitro.

(B) Further studies of the background incidence of diet-induced disease in man and laboratory animals are recommended.

In planning research on the safety of novel foods it must be borne clearly in mind that the scientific community is profoundly ignorant of the risks associated with traditional foods. Doll and Peto in "The Causes of Cancer" (Oxford Univ. Press, 1981), for example, have suggested that in the U.S. 40% of cancer in males and 57% in females is attributable to diet. The precise factors involved are not known. Other diseases may likewise be linked to diet. Therefore the objective of toxicity testing of novel foods is not a search for near absolute safety as with food additives, but an attempt to ensure that novel foods are at least safe as, or safer than, the traditional foods they replace.

In order to identify the toxic properties of novel foods, it is most important to establish the adverse effects on human health of "traditional" foodstuffs both in man and in the animal models used in safety evaluation studies. Human studies should in particular consider the effects identified under (A) above.

(C) Analytical profiles of common foods are needed.

Information on the "unknown" constituents of foods for which there has been extensive human experience is needed in order to identify abnormal structures. This data is required to:

.../27

a) ascertain which constituents in a novel food man has not been previously exposed to;

b) identify components of present foodstuffs which may require some toxicological assessment;

c) improve quality control and product identification criterion.

Food constituents include a wide variety of chemicals usually present in traditional foods at a relatively low level. Some of these constituents display significant toxicity and if ingested at high levels may lead to disease in animals and possibly man. Several publications* describe known toxicants that occur in foods and new suspects are continually appearing in the scientific literature. There is a need to list these food constituents with an assessment of their toxic properties where known and to decide which of them are of sufficient concern to warrant particular study in novel foods.

Research is also needed on methodologies for identifying chemical differences between novel foods and those of the natural food matrix.

(D) Development of effective post marketing surveillance systems.

Because of the large numbers and wide range of population involved, and the considerable potential duration and frequency of exposure which may occur with a novel food, monitoring for related adverse effects is essential. Pilot studies are needed to develop the most effective surveillance systems of novel foods following commercial manufacture and distribution.

(E) Structure/activity relationship for "abnormal" classes of food constituents.

There are several classes of constituent which are commonly considered to be of toxicological concern, e.g. odd numbered carbon chain fatty acids, cyclic fatty acids, long chain hydrocarbons. It would be valuable to assess the actual toxic properties of such materials to enable us to move to a position of prediction for related substances.

* Toxicants naturally occurring in foods: National Academy of Sciences (USA) 1973.

Toxic constituents of plant foodstuffs: I.E. Liener (Academic Press) 1973.

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(F) Early detection of adverse effects in man and animals.

If simple detection methods could be developed for identifying the development of a serious adverse reaction at an early stage, i.e. when any lesion could be remedied by withdrawal of the offending agent a major barrier to the introduction of novel foods would be overcome. These may be biochemical indicators of normal/abnormal functionality, useful at dose levels well below those producing apparent adverse effects. Ideally, the same methods should be equally applicable to man and common animal models.

The most sensible approach would appear to be in the identification of changes in the profile of intermediary metabolism products in biological fluids produced by particular chemically identifiable novel foods. Thus, for example, for a novel carbohydrate the study would focus on changes in key intermediates (particularly at the rate limiting steps) in carbohydrate metabolism. Work would also of course be required to establish the relationship between particular changes and subsequent development of recognisable disease. Once the validity had been established of monitoring such changes as an early indicator of disease, it should be possible to develop appropriate alternative in vitro test systems.

(G) Methods for testing the nutritional quality as well as the safety of novel foods are required. Tests for macronutrients (especially carbohydrates and fats) need to be defined and standardized.

(H) Research is suggested in determining the amount of modified DNA that is released when dealing with genetically modified products. Methods of controlling the release of the DNA to the environment must be investigated.

..../29

CONCLUSION

It is clear from the foregoing that no simple straightforward and inexpensive protocols exist by which to determine the safety of uniquely novel foods for continuous consumption in significant quantities. The evidence strongly indicates, particularly for developing countries which possess a modest scientific resource, that greatest priority be given to traditional agriculture and to the increased production of familiar foods rather than to the synthesis of or conversion of unfamiliar materials into novel foods.

Such methods of assessing safety as are available and are discussed above require extensive and expensive resources, resources not available in many countries.

Finally, under no circumstances should the poor people of the world be encouraged to be the test consumer market for novel foods that have not been demonstrated as safe and which have not and probably would not be considered acceptable by more affluent communities.

SAFETY OF NOVEL FOODS MEETING

APPENDIX I

PARTICIPANTS

PARTICIPANTS

SAFETY OF NOVEL FOODS

Dr. S. Gunner
Health & Welfare Canada
Health Protection Branch
Bureau of Chemical Safety
Ottawa Canada

Dr. G. Sarwar
Health & Welfare Canada
Health Protection Branch
Bureau of Nutritional Sciences
Ottawa Canada

Dr. D. Clayson
Health & Welfare Canada
Health Protection Branch
Bureau of Chemical Safety
Ottawa Canada

Dr. Bruce Holub
Canadian Centre for Toxicology
University of Guelph
Guelph Canada

Dr. Anthony Magnin
Connaught Laboratories Ltd.
Willowdale Canada

Prof. J. W. Bridges
Director
Robens Institute of Industrial
and Environmental Health & Safety
University of Surrey
Surrey England

Dr. Nevin Scrimshaw
Department of Nutrition
and Food Science
M. I. T.
Cambridge Massachusetts
U.S.A.

Dr. Richard L. Hall
Vice President
Science & Technology
McCormick & Company Inc.
Hunt Valley Maryland
U.S.A.

Dr. Sanford A. Miller
Director
Centre for Food Safety and
Applied Nutrition
Food and Drug Administration
U.S.A.

Dr. B. S. Narasinga Rao
Director
National Institute of Nutrition
Jamia Osmania
Hyderabad India

CHAIRMAN: JOSEPH H. HULSE
Vice-President Research Programs
IDRC
P.O. Box 8500
Ottawa, Ontario
K1G 3H9

RAPPORTEUR: DYSON ROSE

IDRC OBSERVERS

A.D. Sauder
Research Officer
Research Programs

J. Hardie
Deputy Director
Office of Planning
and Evaluation

Ann Brown
Office of Planning
and Evaluation

Dr. K. Smith
Associate Director
Maternal & Child Health
Health Sciences Division

Carol Lynn Pass
Program Officer
Maternal & Child Health
Health Sciences Division

A. McNaughton
Program Officer
Cooperative Programs
Agriculture, Food and
Nutrition Sciences Division

SAFETY OF NOVEL FOODS MEETING

APPENDIX 2

PAG/UNU GUIDELINE NO. 6: PRECLINICAL TESTING OF NOVEL SOURCES OF FOOD

PAG/UNU GUIDELINE No. 6: PRECLINICAL TESTING OF NOVEL SOURCES OF FOOD

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Novel foods are defined as those not previously eaten by a human population, and that cannot be considered to be minor processing variants of conventional food. This guideline is directed towards commonly used foods processed by new techniques and to raw materials not so far used as human food either directly or by inclusion in food products. Similar attention needs to be applied to new varieties of conventional foods or those that have been genetically changed. Clear examples for application of the guidelines are new foods developed by isolation from conventional sources by unusual techniques, and yeasts, bacteria, molds, or algae, i.e., the so-called single-cell proteins. It is recommended that all new foods, including proteins, be evaluated with respect to their safety for use and nutritional value before application as a human food source.

It is intended that this guideline serve as a general recommendation rather han as a series of mandatory procedures. The guidelines have been developed in general terms to describe the categories of data that need to be provided in some cases, but not necessarily in all. Thus, processes involving the use of solvent extraction or unusual heating conditions, or the utilization of food additives in a variety of combinations, would not require a full preclinical evaluation despite possible changes in digestion, absorption, or metabolism.

The development of a protocol for a specific food material will depend upon its similarity to a conventional food, the kind of technological process applied in its preparation, and the conditions of its intended use as prepared for consumption. Prior history of safe use may be taken into account in the evaluation of a novel food proposed for general consumption, but this alone is not necessarily sufficient to preclude adequate preclinical testing by currently available, more objective, laboratory animal feeding studies.

The extent of animal testing considered necessary before undertaking trials in human subjects will depend on the degree of novelty of the food. In the event that the observations and results of a preclinical appraisal of a novel food are to be submitted to a regulatory or institutional agency as a basis for clinical trials, it is advisable to review the proposed protocol in advance with such an agency in the interest of saving time and effort. In principle, products intended for incorporation into animal feeds may not require such extensive testing as is suggested for human foods, but Guideline 15 should be consulted for tests considered essential.

The physical and chemical identity of the industrial product should be established to be essentially the same as that of the material tested experimentally. To be truly significant, the studies should be conducted on the product as made on a production scale rather than by laboratory batch. Where this is not possible, the process used to produce the test material and its specifications should not be significantly different from the process and product to be marketed. If the organism or source material has been genetically modified, some of the preclinical and clinical studies may require repetition. However, minor variations in processing conditions need not necessitate repetition of the entire series of preclinical or clinical studies. Attention must be given to contaminants arising from extraction or refining, as well as reaction products resulting from heat processing. Substances used as lubricants or binders (e.g., in texturization) should also be considered in this connection.

When evaluating a new product, the following categories of information are likely to be needed:

1.1 *Specification of the material.* This is a basic requirement; it includes a description of how the product is produced, the results of chemical and microbiological analyses, and details of manufacturing specifications.

1.2 *Nutritional value,* as predicted from chemical composition, with particular emphasis on nutritional value, bioavailability, and digestibility obtained *in vivo.*

1.3 *Sanitation,* with respect to the source of the raw material and the conditions under which it is processed. Hygiene aspects as well as potential pathogenicity, if appropriate, should be taken into account.

1.4 *Toxicological safety,* as predicted from information concerning methods of production, chemical and physical properties, content of micro-organisms and their metabolites. Toxicological studies in laboratory animals may be required. The extent of the preclinical testing programme should be decided for each new product, based on a consideration of its source, composition, and the nature of the process employed in its production. As examples, the species of fish used for the production of fish protein concentrate, the micro-organisms used as single-cell proteins, and the extraction systems employed in processing, may determine how much preclinical evaluation is required. Choice of procedures should be exercised with judgment based on experience. A product intended for use as a milk substitute or a weaning food will demand additional preclinical evaluation compared to products intended for use by older children or adults.

1.5 *Technological and physical properties* from the point of view of incorporation of the product into current-

tly acceptable foods, or the fabrication into new food items.

Tests and procedures to be used are.

2.1 *Chemical analyses* to define the approximate composition of the material, including the presence of contaminants, pesticide residues, solvents, naturally occurring or adventitious toxins, specific additives, and natural components with unusual structure.

2.1.1. *Proximate composition*, i.e., moisture (and total solids), total nitrogen, "fat" (ether extract), ash, crude fibre, and "available carbohydrate".

2.1.2 *Protein*

a) The nitrogenous components should be hydrolyzed and the amino acid spectrum determined. The amino acid composition should be expressed per 16 g N. Since lysine is the principal (although not the only) essential amino acid likely to become bound and thus unavailable as a result of heat processing, the slightly modified Carpenter method is especially useful as a quality control procedure (1).

b) The presence and amount of non-protein nitrogenous components such as glucosamines, amides and amines should be determined, particularly in the case of products derived from animal sources.

c) The content of nucleic acid should be determined in single-cell proteins.

2.1.3. *Fat*

The solvent extract should be analysed for the presence and content of triglycerides, unsaponifiable lipids (steroids) and phospholipids. If the ether extract is greater than 1 per cent, the fatty acid profile should be determined; the ratio of polyunsaturated to saturated fatty acids should also be calculated. Single-cell proteins derived from petroleum hydrocarbons should be analysed for total and carcinogenic polycyclic aromatic hydrocarbons by a suitable quantitative method.

2.1.4. *Ash*

Ash should be analysed for its content of calcium, phosphorus, iron, iodine, alkali and alkaline earth elements, and heavy metals. Products of marine origin should also be analysed for mercury, arsenic, and fluorine. In the light of concern over mercury contamination of fish from lakes, streams, and marine waters, attention should be directed to the possible presence of inorganic and particularly alkyl mercury and cadmium in protein concentrates derived from fish or algae.

2.1.5. *Undigestible Material*

True digestibility should be defined, and the nature of the undigested material ascertained. Examples are cellulose, hemicelluloses, pectic substances, gums, lignin, and some other cell wall-associated substances. While such indigestible material may interfere with the absorption of certain nutrients, it may, on the other hand, be beneficial in a way similar to dietary fibre.

2.1.6. *Vitamins*

Analyses should be conducted for all of the major vitamins except those for which a low lipid content or instability under processing conditions indicates little likelihood of their presence in significant amounts.

2.1.7. *Food Additives*

Food additives used should be declared and levels specified, based on quantitative analysis.

2.1.8. *Processing Damage*

Useful information concerning the effect of heat on the product may be obtained not only by determinations of available lysine, but also be products of the Maillard (browning) reaction. Understanding of the effect of alkali treatment of the product may be obtained by the determination of lysinoalanine.

2.1.9. *Miscellaneous*

Depending upon the nature of the raw material and the conditions employed in its production, special analyses of the product should be conducted for:

a) Solvent residues, such as polycyclic or chlorinated hydrocarbons,
b) Pesticide residues,
c) Naturally occurring toxic substances, e.g., gossypol, haemagglutinins, and marine toxins (it should be noted that there are no satisfactory non-biological tests for the latter category of substances).

2.2 *Microbiological examination* is necessary for viable micro-organisms, both pathogenic and non-pathogenic, aerobic and non-aerobic, vegetative, and spore-forming. In the case of single-cell protein, the microbiological tests should also indicate the taxonomic and potential pathogenic status of the organisms grown, and the sanitary nature of the fermentation or processing conditions. These proteins should contain no viable cells of the producing organisms, thus eliminating any problems of pathogenicity.

2.3 *Nutritional Evaluation*

Gross and available energy should be defined.

The value of a protein product in promoting growth and nitrogen retention may be obtained with young rats or other laboratory animals when fed as a sole source of protein or as a supplement to other foods. The digestibility of a protein product should be determined *in vivo*. For a description of the nutritional evaluation of protein foods, including their energy assessment, see Reference 1, Chapter 5.

2.3.1. *Protein quality* studies may be conducted with young rats or other laboratory mammals, to indicate the value of a protein product for promoting growth and nitrogen retention when fed as the sole source of protein and as a supplement to others foods.

2.3.2. *Digestibility* of the protein product is best determined *in vivo*. Investigations to determine the rate and degree of hydrolysis by pepsin and pepsin plus trypsin *in vitro* may be of value to simulate conditions within the human gastrointestinal tract. Calculations based on the essential amino acid content of enzymic hydrolysates have been adapted for estimating the biological value (utilisation) of proteins.

2.4 *Safety Evaluation*

The identity and reproducibility of the test material must be established with that produced commercially by chemical and other relevant procedures. Safety evaluations using such test materials are based on feeding studies in rodents and other experimental mammals. It is assumed that the data will be required for regulatory approval purposes, and therefore any reports of investigations submitted must include full details and data for control as well as test groups and appropriate statistical analysis of the findings. Brief descriptions of the observations and conclusions are not acceptable. The initial short-term studies should be followed by long-term tests, as appropriate, to define the presence of toxic substances.

Attention should be given to the following:

2.4.1. *Naturally-Occurring Toxic Substances*

Naturally-occurring toxic substances found in plants include carcinogens (e.g., cycad nuts, oil of sassafras), goitrogens (Brassica species), haemagglutinins now called lectins (e.g., phaseolotoxin in legumes), lathyrogens (e.g., vetch, sweet peas), cyanogenetic glycosides (cassava and certain beans and nuts), and estrogens (in seeds and leafy vegetables). Marine sources of protein, such as fish or shellfish and the algae or plankton on which they feed, may contain highly toxic substances. Naturally-occurring toxic agents can be avoided either by care in the selection of the raw materials or by appropriate methods of storage, heat processing, or extraction.

2.4.2. *Microbial Toxins*

Raw materials subject to microbial contamination and spoilage must be examined for the presence of staphylococcal and clostridial toxins. Raw materials exposed to warm, humid conditions that induce fungal growth must be examined for the possible presence of mycotoxins, such as the aflatoxins.

2.4.3. *Residues*

Protein concentrates that have been isolated or refined by means of solvent extraction should be analysed for the possible presence of solvent residues and any products that may be formed, particularly by the use of reactive chlorinated hydrocarbons. In the event that any such residues are present, toxicological data should be available to establish safe limits. Depending upon the nature of the raw materials, the media, and the conditions of processing, analysis for the possible presence of impurities or contaminants such as solvent residues, heavy metals, fluoride, etc., should precede any toxicological feeding studies.

2.4.4. *Nutritional Adequacy of Test Diet*

It is essential to differentiate between nutritional inadequacy and appetite depressant effects, as well as toxicity, all of which can suppress

growth. The basic diet to which test food is added should itself be nutritionally adequate for normal growth and development of the animal species employed. The extent to which the test food may contribute high levels of lipid, carbohydrate, protein, minerals, or indigestible material may create the need for adjustments to balance out these factors between the test and control diets. This applies to most proteins, especially where the amino acid content provided by the test material should be added to basal diet components in order to satisfy nutritional requirements.

Physical form of the diets is important. Experience with laboratory rodents has shown that for a short-term study, i.e., a 3-month test, either a "synthetic" type diet, based on casein and starch, or "natural" diets, based on normal food ingredientes are satisfactory. However, for long-term chronic toxicity or carcinogenicity studies, natural diets are essential.

2.4.5 *Highest Feasible Feeding Level of Test Food*

The nutritional and physical constraints placed upon a food preclude the testing of large multiples of potential-use levels, wich is accepted practice for the safety evaluation of food additives. Nevertheless, the highest dose level practicable should be included, keeping in mind that excessive amounts of high-quality foods may depress growth and feed efficiency. If feasible, graded levels of the test material should be reflected in the experimental design, but it is not realistic to establish a dose-response curve.

2.4.6 *Choice of Animal Species*

The rat is the preferred species. Mice are also used, but less is known of their nutritional requirements, and their size precludes obtaining sufficient blood or urine for examination. Among the non-rodent species, beagle dogs, Rhesus monkeys, and miniature pigs have been used for short-term but not for chronic life-span studies.

2.4.7. *Selection of Animals, Animal Husbandry, Nature and Frequency of Observations*

These procedures should be in compliance with Good Laboratory Practice recommendations. Technical details for conducting individual tests may be found in published international and national guidelines.

2.4.8. *Nature of Studies to be Performed*

For screening purposes, novel food products should be subjected at least to short-term toxicological tests in one rodent and one non-rodent species. They should also be examined for evidence of components with mutagenic potential by the use of a battery of mutagenicity tests in both prokaryotic and eukaryotic systems, looking particularly for evidence of point mutations, chromosomal changes, and inteference with DNA metabolism. The degree of novelty of a potentially important food item (both source and method of production) should determine the need for definitive studies. Among these, reproduction and lactation studies are important. While they may not be indicated in the case of a fish or cereal protein concentrate, such tests should be included in a protocol for safety evaluation of single-cell protein. These tests should extend to at least two filial generations, and could be continued with teratology and dominant lethality studies. If necessary, the F_1 generation could be used for chronic toxicity and carcinogenecity studies.

2.4.9 *Statistical Analyses and Interpretation of Findings*

In the interpretation of the responses to toxicological tests, the statistical significance of differences of responses between test and control groups plays an important role. Hence, the size of experimental groups as well as the quantitative rating of both objective and subjective observations are particularly relevant.

However, whatever statistical probability is adopted as the basis for defining significance, the chance that a single group may deviate from the norm without actually indicating a biological aberration cannot be ignored. Judgment founded on experience of the investigator and past performance of the particular strain and colony of animals must be given due weight. Interpretation of experimental findings should take into account the quantitative relationship of the experimental test levels versus use conditions of the product under investigation, interspecies variations, the limited number and variety of observations incorporated into the safety evaluation programme, and the relative size of the test and human populations.

REFERENCE

1. Peter L. Pellett and Vernon R. Young (Eds.), *Nutritional Evaluation of Protein Foods*, The United Nations University World Hunger Programme *Food and Nutrition Bulletin* Supplement 4, Tokyo, 1980, p. 95.

SAFETY OF NOVEL FOODS MEETING

APPENDIX 3

PAG/UNU REVISED GUIDELINE NO.7: HUMAN TESTING OF NOVEL FOODS

PAG/UNU REVISED GUIDELINE No. 7: HUMAN TESTING OF NOVEL FOODS

INTRODUCTION

Novel foods are defined as those that have not been eaten before by the population for which they are intended, or at least not in significant amounts. Similarly, novel processes applied to traditional foods require examination, as do new varieties. After preclinical testing for possible toxic constituents, the features that require examination are nutritional value and components that may cause dietary intolerance.

The tests outlined in PAG/UNU Revised Guideline No. 6, "Preclinical Testing of Novel Sources of Food" (1), should provide adequate information on the safety of a product before commencing any testing with human beings. Human testing, as outlined in the remainder of this Guideline, should only be carried out after a complete examination of the features defined in PAG/UNU Guideline 6. Although the toxicological tests outlined should be performed for very novel foods or novel processes applied to traditional foods, there is a real danger of excessive and unnecessary experimentation with minor variations in formulas using previously tested ingredients or processes.

If animal tests reveal any toxicological problems, these should be resolved before the material is fed to humans. Examples are substances such as lectins (haemagglutinins), trypsin inhibitors, and cyanogenic glycosides.

Other animal tests will reveal the available energy content of the food, digestibility and quality of the protein, and availability of minerals, thereby providing information on the nutritional value of the product that will complement the information from analysis of carbohydrates, lipids, and fatty acid composition, proteins, and amino acid composition as well as vitamins and minerals.

Nutritional measurements on human beings are subject to such individual variation thay they rarely contribute as much useful information as the measurements of nutritional value in experimental animals. They may, however, be desirable in certain instances, such as when comparing the relative value of two sources, or substituting a novel source for a traditional one. In such cases it is necessary to establish precisely what information is required and then to select from the tests described below those most likely to supply the answer to the appropriate questions.

In general, it follows from first principles that any safe food that supplies significant dietary energy, protein, and other nutrients will be of value to the consumer. However, the acceptability of a food, or tolerance to that food once ingested, cannot be foretold from animal experiments alone, and hence human testing is a prerequisite.

CATEGORIES OF NOVEL FOODS

Products that require full testing, both preclinically and clinically, are:

1. Food sources not previously consumed by human beings, or at least not in the amounts proposed.

2. Products previously accepted that have been subjected to such different processing conditions that questions can be raised regarding their nutritional or toxicological properties.

3. In certain instances, new varieties of foods and foods newly introduced to a population, even though they have already been consumed safely in other areas.

It is assumed that the following information will be available in full detail before proceeding with human testing:

1. Availability of supplies of the novel food, including aspects of its distribution and shelf-life.

2. The mode of consumption, i.e., whether it is to be incorporated into other foods or dishes, used a supplement, fed alone (as it may well be in the case of infant foods), or used as a substitute for traditional foods, and whether it is to be cooked after distribution.

3. Full microbiological data pertaining to public health hazards.

Particular care should be taken to ensure that the material used in the tests is truly representative of the novel food that will ultimately be marketed. Whenever possible, the same large-scale industrial process that will be used to produce the novel food for marketing should be used to produce the food used in testing, since the specification of food produced in a pilot plant may differ significantly from that produced commercially. Where there is an inherent risk of variability of the product – e.g., in yeast products – stringent batch control after general production will be

necessary to ensure that the food as marketed does not differ from that tested. Single-cell protein preparations may need to be examined by suitable screening tests that should be applied after each process modification. It is also important to consider the form in which the food will be tested. Food that would normally be cooked prior to consumption should be treated in a similar manner before feeding or testing *in vitro*.

NUTRITIONAL TESTING IN HUMAN BEINGS

The approximate nutritional value may be determined by chemical analysis. If the food is to be consumed processed or cooked, then the nutritional tests should be carried out on such cooked foods. For testing nutritional value *in vivo*, the food should be fed under the conditions it is expected to be consumed in practice, i.e., mixed with the rest of the diet in amounts likely to be used and not at higher levels. Time and effort should not be devoted to measurements on the isolated foodstuff at excessive levels when it is not intended to be consumed alone, and to measuring parameters such as conventional net protein utilization that have little relevance.

Methodologies for determining protein value through analytical procedures and experimental animal and human trials are given in the UNU Food and Nutrition Bulletin Supplement No. 4 on *Nutritional Evaluation of Protein Foods* (2). Approximate protein value can be established by determination of essential amino acid composition and calculation of a protein score. If human feeding studies for this purpose are desired, procedures are available, although they are time-consuming, costly, and add little to the information on protein value obtained from laboratory analysis and feeding experimental animals. It is, however, desirable to conduct a five- to ten-day nitrogen balance trial to measure the product's digestibility by human subjects.

If the food is intended for infant feeding, selected anthropometric parameters can be measured over a period of at least three months. These can be limited to weight and height increment, head and arm circumference, and triceps skinfold. Other possible parameters include serum and urinary urea, prealbumin, albumin, transferrin, ceruloplasmin, cholinesterase activity, aspartate aminotransferase (formely SGOT), hydroxyproline, creatinine-length index; and for vitamins, it may be desirable to measure the usual enzyme-reactivation tests and other common nutritional indices.

CRITERIA OF TOLERANCE

It must be borne in mind that a significant percentage of individuals is intolerant to one or more traditional foods. It has been stated that there is no food that is wholly free of adverse effects in at least an occasional individual. Most individuals have ascertained by trial and error which foods cause them ill effects when these effects follow fairly rapidly after the ingestion of the offending food. Recent reports from the United Kingdom suggest that about 30 per cent of the population sampled in some countries respond adversely to one or more foods, and up to 3/4 per cent avoid one of the major traditional foods widely consumed by the population as a whole (3). The frequency of allergic responses to milk protein alone is variously reported as up to 8 per cent (4), and allergies to chocolate, corn, legumes, eggs, citrus fruit, tomatoes, wheat, and pork are also very common (5). Allergic responses call for special consideration and can be specifically examined and characterized by the presence of antibodies. Gastrointestinal intolerance to ingested food will be indicated by loss of appetite, flatulence, undigested stool contents, diarrhoea, and abdominal pain. Cutaneous rashes are also quite common (6).

PRELIMINARY ACCEPTABILITY AND TOLERANCE TESTS

When novel foods are developed, it is essential that their acceptability be evaluated in human subjects under controlled conditions before they are used in an unsupervised manner. A variety of adverse reactions may be observed in humans that are undetectable and unpredictable in experimental and farm animals even with extensive testing.

The most common symptoms to be looked for in human subjects are those of gastrointestinal intolerance. In addition, various allergic reactions may occur in some individuals after consumption of almost any common food, especially if it is a protein source. It is the frequency of such reactions that must be evaluated rather than their expected absence. Moreover, symptoms may be due to psychosomatic factors.

Because of these factors, it is important to feed a control group simultaneously. Ideally, the trial is conducted so that individuals are randomly assigned to experimental and control groups, stratified by sex if necessary, in a double-blind cross-over design. Only after a low frequency of adverse symptoms is assured can additional trials be conducted without the necessity of a control group. Once again, the inadvisability of high-level testing must be emphasized if the product is unlikely to be consumed at such levels; mistaken conclusions may be drawn from reactions at high-intake levels even with traditional foods.

SUBJECTS

In general, 25 to 50 human subjects can be studied initially. All subjects should be in good health, as determined by medical history, physical examination, and routine blood and urine tests. Individual medical histories should focus on a history of personal or family intolerance to specific foods and manifestations of allergy, such as asthma, hay fever, and urticaria. However, no individual should be excluded on this account, since it is essential to determine the prevalence of symptoms to the novel food in the general population for whom it is intended. The purpose of the study, the cooperation required, and potential risks involved should be explained fully to each subject. Protocols should be reviewed by the multidisciplinary group established by the institutions to evaluate such studies using humans as experimental subjects. In addition, subjects should sign appropriate consent forms. For subjects who are legally minors, written parental or guardian consent must be obtained.

The actual numbers of individuals involved in the study will be a function of the preliminary data. True allergic responses will be characterized by antibody formation and will potentially require fewer participating individuals than in those cases where intolerance is a problem. In the latter case, this should be tested in a manner similar to that used for bacteriological sterility; that is to say, if any subject responds adversely, then the tests should be repeated on larger numbers of subjects to ascertain numbers that may be intolerant. So, while 20 to 50 subjects may be an adequate number for general purposes if intolerance is revealed, this number may need to be increased.

STUDY DESIGN

Subjects should consume both the novel and a control food, preferably one that is already commercially available, in a double-blind cross-over study design. Subjects should be allowed complete freedom to consume their usual diet. Since this will vary and may include meals or foods that in themselves cause symptoms, it is essential that each subject keep a diary of all significant departures from the usual in his or her daily diet and activities. These often prove of value in the retrospective interpretation of the results of a tolerance trial.

An appropriate study period would be two four-week periods separated by a one-week interval. In the first four-week period, subjects ingest daily either a fixed amount of the control or experimental material. After completing the initial four-week phase, subjects will return to their regular diet without supplementation. Thereafter, the second four-week period will be started, and subjects, unaware of the type of supplement they are taking, will begin ingesting the second protein. Administration of the material for five to six days each week for four weeks is adequate. Experience indicates that individuals not developing symptoms in the first 20 days are not likely to do so thereafter.

METHOD OF ADMINISTRATION

Any method of oral administration that facilitates a double-blind trial is acceptable. For example, the material can be incorporated into cookies, cakes, pudding, or other acceptable supplements to a usual diet if it can be introduced so that its presence cannot be specifically identified. Novel foods in powder form can often be tested by allowing the subjects to mix them into bouillon or any of a variety of fruit juices according to individual preference, and they can vary their choice from day to day. In this case, however, there must be available a similar control material for which tolerance is already well established. Experienced nutritionists can usually find an acceptable method of administration.

All subjects recruited should have the opportunity to taste the materials to be consumed before making a commitment to the trial. If this is not done, a number of subjects are likely to confound the trial by dropping out in the first few days because they do not like the taste, texture, smell, or some other aspect of the substances offered.

LEVEL OF FEEDING

Tolerance studies should be designed taking into account the intended use of the substance. For example, yeast as a vitamin supplement may be consumed at less than 5 grams daily, as a functional food additive rarely more than 10 grams, but as a significant protein supplement, the amount might be 30 grams or more, providing it does not supply more than 2 grams of nucleic acid. If the amount intended for testing is more than 15 to 20 grams per day, it should be divided into two or more feedings per day. If a supplement is intended to be added to a traditional staple like bread or a rice dish, then it should be given daily or even more frequently, in relatively large amounts, following normal dietary practice so that the experimental material is fed at the upper intended limit of use, which may involve daily administration of 30 grams or higher.

For a totally new food source it may be more prudent to conduct a pilot trial with a smaller number of subjects at a lower level. Materials intended only as minor additives to a mixed diet may be tested at levels as low as 5 to 15 grams daily. When evaluating the data, two contrary effects may be noted: intolerance to which the subject soon becomes adapted, and boredom from eating the same food over a prolonged period. The former (as in flatulence) can be examined by introducing the

food in small and increasing amounts over a few days. The latter will always be true even for attractive traditional foods if eaten too frequently.

LEVEL OF DIETARY ENERGY INTAKE

The level of dietary energy intake should be sufficient to maintain constant weight in adults or adequate weight gain in children.

OBSERVATIONS TO BE MADE

Before subjects begin the feeding trial, initial blood tests are required. These should include haemoglobin or haematocrit, white blood count, and a peripheral blood smear to determine the percentage of the white blood cell components. In addition, serum electrolytes, blood urea nitrogen, creatinine, and serum uric acid should be obtained. Tests of liver function should be included; i.e., albumin globulin, bilirubin, aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT). Serum calcium and phosphorus should also be determined. A urine sample should be obtained for complete analysis of cells, protein, and pH.

These blood and urine tests should be repeated at the end of the study or sooner in subjects dropped from studies because of adverse reactions. It is important to ascertain if any of these biochemical parameters change during the acceptability study. Examination of the serum immunoglobulin levels is necessary in individuals with possible allergic reactions.

Further, to assure that the novel food is not associated with unpleasant or adverse reactions, all participants in the study should be asked to keep a diary as noted above that will note changes in mood, appetite, sleep patterns, libido, and other subjective reactions.

UNNECESSARY TESTING

Much time and effort has been devoted in the past to measuring such parameters as protein quality (by PER or NPU) in human beings. This is not necessary for two reasons:

1. Results obtained in closely controlled laboratory tests are often not repeatable under field conditions. This would indicate that such parameters are unimportant under normal, free-living (field) conditions.

2. Most such measurements have been carried out on the food alone or on protein isolates, whereas it will be consumed mixed together with other foods. Consequently, the protein quality of the food alone is not of any interest. For example, a protein source completely deficient in one essential amino acid might well be rejected as the result of such tests, whereas if the missing amino acid is not limiting in the mixed diet, then that protein may make a valuable contribution to the diet.

GENERAL CONSIDERATIONS

It follows from first principles that any safe food that supplies additional energy, protein, and other nutrients will be of value *even if it is not possible to demonstrate such benefit in the trials.* While it may be necessary in order to convince policy makers and governments to demonstrate the benefits of the novel food (in which case growth, nitrogen balance, skinfold increases, etc. may need to be measured and reported), it is not necessary for the nutritionist. While he would often like to quantify the benefits of the supplement, such measurements are open to enormous individual variation and rarely have any real meaning. The only instances where such measurements *may* be of value are when different sources of nutrients are being compared, and even then the variability of the findings usually vitiates the results. Economic and palatability considerations frequently outweigh all such technical detail of nutrient differences.

For example, while weight gain is the best measure of the value of a supplementary food, such measures require prolonged periods of testing (several months) and show the most marked results in children who were previously poorly nourished. Such methods cannot be applied to adults. Any supplement that provides additional energy and nutrients will be of value when the diet has been poor, and testing the precise effects of such supplements is often quite superfluous and costly.

Finally, once the use of a product has commenced, it is advisable to have a mechanism by which any adverse reactions may be monitored in order that corrective change may be introduced.

REFERENCES

1. PAG/UNU Guideline No. 6, "Preclinical Testing of Novel Sources of Food," The United Nations University *Food and Nutrition Bulletin* 5 (1): 60 (1983).
2. P. L. Pellet and V. R. Young (Eds.), *Nutritional Evaluation of Protein Foods*, The United Nations University *Food and Nutrition Bulletin* Supplement No. 4 (The United Nations University, Tokyo, 1980).
3. A. E. Bender and D. R. Mathews, "Adverse Reactions to Foods," *Brit. J. Nutr.* 46 (3): 403 (1981).
4. S.I. Bahna and D. C. Heiner, *Allergies to Milk* (Grune and Stratton, New York and London, 1980).
5. F. Speer, *Food Allergy* (PSG Publishing Co., Inc., Littleton, Massachusetts, 1978).
6. S. Achinewu, "Food Allergy and Its Clinical Symptoms in Nigeria," The United Nations University *Food and Nutrition Bulletin*, Vol. 5, (3) (to be published, 1983).

SAFETY OF NOVEL FOODS MEETING

APPENDIX 4

PAG/UNU GUIDELINE NO. 12:

THE PRODUCTION OF SINGLE-CELL PROTEIN FOR HUMAN CONSUMPTION

PAG/UNU GUIDELINE No. 12: THE PRODUCTION OF SINGLE-CELL PROTEIN FOR HUMAN CONSUMPTION

Reprinted from: *Food and Nutrition Bulletin*, Vol. 5, No. 1, 1983

PAG/UNU GUIDELINE No. 12: THE PRODUCTION OF SINGLE-CELL PROTEIN FOR HUMAN CONSUMPTION

The following is presented to supplement PAG/UNU Guidelines 6 and 7 as a general guide for producers on processes for, and products from, single-cell protein (SCP). Because of the large number of potential organisms and processes, a complete description of any individual process is beyond the scope of this Guideline.

1. INTRODUCTION

SCP is defined here as a biomass of yeasts or other fungi, bacteria, and algae to be produced and/or processed as food for human consumption. Such food in addition to protein has a significant caloric and other nutrient content, e.g., vitamins. The nutritive value of the protein component is of particular importance if the product is to be used as a protein supplement

The traditional concept of "good manufacturing practice" should apply in the sense that it is currently used in the food industry. Plants and equipment should be of sanitary design, and special care should be exercised in all aspects of processing, including raw material selection, quality control, sanitation, handling, and packaging.

2. TYPES OF ORGANISMS

Various types of organisms have been considered as potential sources of SCP and appear to be capable of meeting the criteria established in this Guideline. It is preferable to select an organism of a species known not to produce pathogenic or toxic variants.

To comply with non-pathogenicity requirements, the final product should contain no living cells derived from the fermentation process. Each individual strain eventually should be evaluated under the exact condition proposed for industrial production.

3. PROCESSES

3.1 *Raw Materials*

Potential carbon sources include carbohydrate-containing materials (molasses, various sources of starch or other polysaccharides, cellulose, whey, sulfite liquor, etc.), various classes of hydrocarbons (methane and longer-chain normal alkanes), alcohols (methanol and ethanol), organic acids, and carbon dioxide. In view of the variety of carbon substrates, attention must be directed to the composition of the media from the standpoint of the possible presence of chemical components (e.g., polycyclic aromatic hydrocarbons from petroleum) that are regarded as health hazards. In addition to the carbon substrate, other materials involved in the process, include sources of nitrogen, buffer salts, minerals, vitamins, air or oxygen, and many contain chemicals such as anti-foam agents, detergents, and flocculants. No material should be introduced into the process that cannot later be removed from the SCP product if necessary to meet safety requirements.

3.2 *Process Variables*

Operating variables of fermentation, such as temperature, air or oxygen supply, pH, cell growth rate, and cell concentration must be carefully controlled to ensure the required product quality and uniformity. The operations following cell production should also be monitored to ensure the required product quality and uniformity. In general, this will necessitate careful and continuous monitoring throughout the course of production and an understanding of the influence of these variables on the composition of the product.

3.3 *Quality Control*

In addition to the quality control procedures associated with factors listed under point 5 (nutritional evaluation), careful attention should be paid to the maintenance of the integrity of the original strain of the organism. An appropriate series of microbiological and biochemical tests should be worked out both to demonstrate the stability of the organism and the absence of undesirable contaminants.

4. SAFETY EVALUATION

The procedure for safety evaluation is outlined in

PAG/UNU Revised Guideline No. 6, "Preclinical Testing of Novel Sources of Food." Following this testing, thorough clinical evaluation is particularly necessary because of effects such as allergenicity, uricogenesis, subjective responses, and other reactions that can be determinend only in humans. These tests area outlined in PAG/UNU Revised Guideline No. 7, "Human Testing of Novel Foods."*

5. NUTRITIONAL EVALUATION

The nutrition quality of the SCP is to be determined for protein and for other nutrients, as recommended in the above-mentioned PAG/UNU Guidelines.

6. COMPOSITION

6.1 *Protein*

Crude protein is total N x 6.25. Total N includes non-protein N and non-amino acid N, such as nucleic acid, urea, amines, and ammonia. The N conversion factor for true protein depends upon amino acid composition. Because of these variables, true protein should be determined by amino acid analysis. As an approximation, corrected protein N can be calculated by subtracting 1.4 x purine N from crude protein N.

6.2 *Nucleic Acids*

There is a limitation to the amount of nucleic acids that should be introduced into human diets, because nucleic acid purines are excreted by man principally as uric acid. In susceptible individuals elevated levels of serum uric acid increase the risk of gout, and increased urinary concentration of uric acid may result in the formation of uric acid calculi (1).

The currently available information (2,3) suggests that there should be not more than two grams of nucleic acid per day introduced into the diet by SCP for adults, and correspondingly less for children, depending on their weight. Because of variations in the nucleic acid content of the biomass of different species, the nucleic acid content can be most usefully expressed as per cent of product

6.3 *Lipids*

Total lipids should be analysed. If lipid content is greater than one per cent, the fatty acid profile should be determined. Triglycerides, phospholipids, and steroid values need to be expressed.

6.4 *Ash*

This should be analysed for minerals of biological importance such as iron, iodine, alkali, alkaline, earth, and heavy metals. The bioavailability of the minerals of nutritional importance can be determined only by measurements in human beings.

6.5 *Dietary Fibre*

Non-digestible materials should be analysed and taken into account in the determination of energy value of SCP. The effect of any such materials on the availability of essential nutrients from the remainder of the diet should be determined.

TABLE 1. Suggested Limits for Viable and Contaminating Micro-organisms

Micro-organism	Number per Gram
Viable bacteria	100,000
Viable yeasts and moulds	100
Enterobacteriaceae	10
Salmonella	1 per 50 g
Staphylococcus aureus	1
Clostridia, total	1,000
Clostridia perfringens	100
Lancefield Group D *Streptococci*	10,000

6.6 *Solvent Residues*

These should not exceed those compatible with good manufacturing practice. Methods of analysis are given in the 14th Report of Joint FAO/WHO Expert Committee on Food Additives,

* Forthcoming

FAO Nutrition Meetings Report Series No. 48, and accompanying monographs, including those for determining residues in foods.

7. SANITARY ANALYSIS

The microbial standards should comply with Table 1.

REFERENCES

1. PAG Ad Hoc Working Group Meeting on Clinical Evaluation and Acceptable Nucleic Acid Levels of SCP for Human Consumption, Geneva, February 1975.
2. J.C. Edozien, "Yeast for Human Feeding; New Data on Safety." FAD/WHO/UNICEF Protein Advisory Group, United Nations, PAG Document 2.23/1 (United Nations, New York, 1969)
3. D. H. Calloway, "Safety of Single-Cell Protein." FAD/WHO/UNICEF Protein Advisory Group, United Nations, PAG Document 2.23/2 (United Nations; New York, 1969).

SAFETY OF NOVEL FOODS MEETING

APPENDIX 5

PAG/UNU GUINELINE NO. 15: NUTRITIONAL AND SAFETY ASPECTS OF PROTEIN SOURCES FOR ANIMAL FEEDING

PAG/UNU GUIDELINE No. 15: NUTRITIONAL AND SAFETY ASPECTS OF PROTEIN SOURCES FOR ANIMAL FEEDING

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PAG/UNU GUIDELINE No. 15: NUTRITIONAL AND SAFETY ASPECTS OF PROTEIN SOURCES FOR ANIMAL FEEDING

1. PROBLEMS AND ISSUES

The need to expand the world supply of protein for human food and animal feed has been well documented. The basic problems are:

a) Increasing worldwide demand for protein.
b) An immediate need to upgrade proteins in many areas of the world.
c) The demand created by both agriculture and industry to modify existing protein supplies and produce new sources and forms of protein.
d) Demands by many governments to evolve objective regulations controlling the quality and safety of modified and novel protein sources, such regulations to be capable of harmonization at the international level to the greatest possible extent.
e) The need to allow unrestricted and unimpeded international export and import of such products, which will require international similarity of national regulations.

These developments have several implications for the future. They imply a possible revolution in animal and human feeding, as people will consume meat, milk and eggs from animals receiving new forms of protein in their feed rations. Before very long, increasing numbers of human beings may receive such protein foods as direct components of their diet. Thus, there is a need to ensure that the new proteins will have a beneficial effect on the nutrition and health of intermediate and final consumers.

After these Guidelines were initially written, a series of single-cell proteins were evaluated using the criteria as originally defined. Their safety, utility and efficiency have been clearly demonstrated. The products have been manufactured and no hazards were detected in the original experimental work or in subsequent commercialisation of these products.

Experience has shown that it is necessary to establish acceptable criteria for determining the nutritive value and safety of protein materials in relation to their use in the rations of the particular animal species concerned, and to consider also the public health implications resulting from the use of the same animals for human feeding.

The PAG/UNU has well-developed Guidelines for the preclinical testing of novel food in laboratory animals (PAG/UNU Guideline No. 6), and for their clinical evaluation in human subjects (PAG/UNU Revised Guideline No. 7).* These Guidelines were stimulated originally by the potential direct use by human beings of microbial biomass grown, in some cases, on unconventional substrates such as hydrocarbons and simple alcohols.

The concepts expressed in PAG/UNU Revised Guideline No. 6 are also relevant to an animal feeding situation, but the need to use laboratory animals as models for testing new food components is removed because of the ability to test directly in the "target species" —the domestic and farm animals for which they are intended. Such experiments are of equal, if not greater, importance than those obtained from conventional experimental animals. However, this does not imply that the latter, which are the biological tools of the toxicologist and nutritionist, cannot provide information relative to the consumption of animal products by humans.

2. THE NEED FOR GUIDELINES

The question arises as to the necessity or usefulness of establishing guidelines for testing protein sources proposed for animal feeds in addition to those already established for preclinical evaluation of novel foods for human consumption. The guidelines have been useful to industrial producers of single-cell proteins, e.g., selected yeasts, bacteria, etc., as the companies required an internationally and mutually agreed upon standard by which both they and governments could gauge the quality, uniformity, nutritive value, and safety of their products. The need to maintain this is still apparent, as is the need to evaluate more conventional feed protein sources produced with the aid of extensive processing systems.

The material to be evaluated initially cannot be derived from full-scale plant production; testing and product acceptance must therefore involve products from pilot plants. Confirmation that the pilot-scale product is similar to the final product is also required.

Many factors must be considered for prior approval of protein sources, including the quality of the raw materials, the acceptability of the critical processing techniques affecting nutritive value, safety, purity, and uniformity of the ultimate commercial

* Forthcoming

output compared with the pilot plant product previously evaluated and approved.

In the case of SCP products, the genetic stability of the micro-organism subjected to continuous fermentation must also be considered. This requires careful development of physical, chemical, and microbiological specifications as well as nutritional and toxicological measurements appropriate for the target species.

The extent to which it is necessary to repeat toxicological and nutritional studies on micro-organisms that have been genetically engineered will depend on the nature of specificity of the DNA interchange between the vector and the host and an understanding of the relative safety of the original vector donor.

3. THE PURPOSE OF GUIDELINES

As stated in PAG/UNU Revised Guideline No. 6, it must be understood that a published guideline does not constitute a compendium of mandatory tests that, unless completely and successfully satisfied, would preclude acceptance of any new food component. Indeed, with the accumulation of experience in routine testing of these products and their feeding to livestock under practical conditions, it seems likely that more informative and possibly more routine test procedures will emerge that will permit improved testing efficiency and economy. It is recognised that toxicologists and nutritionists experienced in these matters should have the prerogative to decide on the extent and design of experimental protocols.

For the guidance of those who may lack the necessary resources in these highly specialised disciplines, it is considered useful to suggest procedures to support acceptable conclusions as to both functional utility and safety of protein sources. Many aspects of the criteria described in PAG/UNU Revised Guideline No. 6 are applicable to proteins intended for animal feeding; particularly relevant are the sections dealing with chemical, physical, and biological evaluations.

However, the criteria for the prediction of protein quality of foods used in human nutrition, e.g., bioassays for protein efficiency ratio, net protein utilisation and biological value, while of interest, are not directly applicable to animal nutrition. This explains the importance of establishing the quality and safety of feed ingredients for nourishment and productivity directly in the target species. Products intended for incorporation into animal feeds may not require as extensive testing as that suggested for human foods, but foods derived from animal sources must be considered from the viewpoint of the possible presence of residues in meat, milk or eggs transmitted from animal feeds.

4. TERMS OF THE GUIDELINE

When reviewing the elements of this Guideline, reference should first be made to the introductory pages of PAG/UNU Revised Guideline No. 6, particularly those dealing with the categories of information needed (1.1 — 1.5) and evaluation procedures (2.1 — 2.4). With respect to ingredients intended for animal feed purposes, the Guideline covers aspects specifically applicable to the feed requirements of the species concerned as well as aspects applicable to foods derived from these animals and intended for human use. With respect to the species involved, the Guideline provides for the evaluation of the quality and efficiency of the protein for maintenance, growth and reproduction, its safety for the target species, and its effect on productivity from the economic standpoint. With respect to foods derived from these animals, consideration of acceptability in terms of flavour, colour, texture, etc., would have been taken into account before marketing any such product. Safety for humans would involve the possibility of contaminants or residues arising from processing or source materials, e.g., substrate or media employed in the case of SCP. In general, protein molecules themselves would be unlikely to present a hazard. The quality and safety of food from animals fed unconventional protein should be predictable from the nutritional and toxicological studies of the protein in the animals fed.

Because of the impracticality of identifying, by means of animal feeding studies, the minute traces of residues or contaminants that might be transmitted from the feed into meat, milk or eggs, detection of any such substances that might be suspected to be present must depend on highly sensitive chemical analytical procedures. The feeding of meat/fat from feed animals to laboratory animals to assess toxicity has been suggested as a means of gaining an insight into the residues of animal products. Experience has shown that, although such protocols might identify very potent toxic or pharmacologically active substances, in all other cases the dilution factors involved in the passage of these supplements through the food animals are so vast as to make these kinds of study relatively meaningless.

In view of the fact that some types of protein sources may be produced in the form of microbial biomass under industrially controlled conditions using agricul-

tural or industrial waste, including both human and animal sewage, special attention must be directed toward ensuring the absence of pathogenic organisms and metabolites (PAG/UNU Guideline No. 12), as well as toxic residues of pesticides or drugs. In the case of inocula for SCP production from any substrate, the micro-organisms selected should be non-pathogenic, notwithstanding the fact that the fermentation process need not involve the use of pure cultures grown aseptically.

After the processing conditions have been established to ensure uniform quality, safety, and nutritive value of the final product, chemical criteria should be determined, as suggested in Table 1.

Sensitive, quantitative analytical procedures must be used to determine the levels of any undesirable residues that could arise from the source or media from which the proteins are derived, or from solvents and other agents employed in any stage of processing. For in-plant control, the methods of examination and analysis should be capable of giving valid, reproducible results.

5. NUTRITIONAL AND SAFETY EVALUATION

The nutritional value of novel proteins for animal feeding should be determined in the species for which its use is intended and as far as possible under simulated practical conditions. In experimental diets conventionally employed for evaluation in rats, the test protein is incorporated as the sole source of nitrogen. Since new protein sources would be used in animal feed as a supplement or partial replacement of soybean, fish meal, or other protein component of the total ration, test diets should reflect likely local, practical rations. Occasionally it may be necessary to effect a total protein replacement, but supra-high protein levels resulting from high test product incorporation should be avoided, as these could introduce irrelevant effects of no significance in the evaluation of safety.

To achieve the best utilization of protein sources used in animal feeds, consideration may be given to supplementation with synthetic amino acids essential for the species in question; e.g., lysine for pigs and methionine for poultry.

In the design of experimental rations for nutritional or toxicological evaluation, care must be exercised to ensure that normal dietary requirements for the species in question are satisfied in order not to confuse nutritional effects with toxic effects. Dietary deficiencies or imbalance may yield apparent toxicological symptoms that are not due to the test material as such, e.g., mineral imbalance causing nephrocalcinosis in rats, or impaired growth response.

TABLE 1. Suggested Criteria for Defining Protein Sources to be Used for Animal Feeding

For All Novel Protein (Dry Weight Basis):
Total nitrogen, full amino acid profile, nucleic acid, and other non-amino components
Total lipid, fatty acid profile, glycerols, phospholipids
Carbohydrates
Fibre content
Ash, inorganic components
Trace mineral components (beneficial: Co, Fe, Zn, Mn, Cu) (harmful: As, Hg, Pb, Cd)
Vitamins
Gross and available energy values
Residual substrates, processing aids
Residual harmful components (e.g., benzo(a)pyrene,* nitrosamines)
Availability of nutrients should be defined where possible

* If specific carcinogenic polynuclear aromatics are found, this might need additional testing

6. TEST SPECIES

In considering the test species for toxicological testing, there are two major points of interest: first, human beings will consume the animals fed the protein, and second, the health and well-being of the recipient animal. Regarding the former, there is the ideal situation of identifying all the constituents of animal products so as to be assured of their safety.

However, the technical difficulties manifest for the products under consideration make it necessary to adopt indirect means. Therefore, the types of studies that are relevant to establish preclinical safety are also suited to this context (PAG/UNU Revised Guideline No. 6). Therefore, as there is an intermediate host whose well-being can also be examined in this type of study, the classical laboratory animal studies can be less extensive unless the data obtained suggest that there is such a need. When considering the choice of animals, a rodent species (preferably the rat), and a non-rodent mammal (which may be one of the target species) are generally employed. As there is emphasis on biological quality as well as toxicological safety, the feeding studies with the target species (e.g., chickens, pigs, fish, calves, etc.) are of considerable value.

7. PROTOCOL FOR EXPERIMENTATION

7.1 *Laboratory Animals*

Reference should be made to PAG/UNU Revised Guideline No. 6, Section 2.4 for an outline of the basic method for safety evaluation of novel foods in rats. The studies should be conducted with groups of experimental animals of sufficient size to yield statistically significant results. Physical inspection of the animals, including observations of weight changes, feed conversion, gross and histopathology, haemocytology, and blood and urine chemistry should be conducted at appropriate intervals.

Reproduction studies in a suitable species are considered essential, extending for ar least two generations.

When there is evidence to suggest the need to do long-term studies to establish the absence of potential carcinogens or chronic toxicity due to cumulative effects, these should be done in at least two laboratory species.

7.2 *Target Species*

The ultimate tests of the nutritional value for animal feeds are the practical trials in which growth, feed efficiency, reproduction, health, survival, and productivity are the bases for evaluation. Short-term feeding studies are only minimal pre-requisites for the introduction of protein sources. Because of the short duration of such tests relative to the tqtal life span, it is not possible in these tests to observe evidence of long-term (chronic) or cumulative effects. Thus, studies for reproductive performance and for carcinogenicity are required where substrates or processing adjuncts suggest the need. If the proposed feed ingredient is to be incorporated in the ration of breeding animals, the appropriate reproductive data should be collected. Where the target species serves for the non-rodent mammal in toxicological studies, biological data should be similar to those gathered from the rat.

8. REGULATORY ASPECTS

Information concerning certain proprietary process details may be required to provide assurance of safety and to ensure adequate quality control as well as uniformity of the final product. Examples of process details that should be reported in the case of single-cell protein production are the nature and properties of the micro-organism, the qualitative composition of the substrate raw materials employed, including major nutrient supplements, and the agents used for special purposes, such as defoamers, emulsion breakers, etc. General processing conditions, such as fermentation method, extraction processes, concentration procedures, drying methods, etc., should be described to the extent needed. Similar criteria for other protein sources will need to be described for each product.

Conformity of the commercial product with the registered product should be based on appropriate sampling of products in the manufacturing plant or in commercial channels. The chemical and biological testing procedures, therefore, will include general methods applicable to all protein sources and feed in general, and to methods selected, especially to relate to the registered product. Certain data to establish criteria of identity should be included in label information.

Approval of a protein source would require statements within the submission detailing nutritional quality, and all safety criteria as outlined within this document. Changes in processing that might affect these features must be reported before the changes are effected.

Chemical, nutritional, and microbiological quality characteristics referred to in this document may be found in PAG/UNU Revised Guideline Nos. 6, 7, and 12. Requirements for additional or improved analytical methods for evaluating novel proteins will be referred to appropriate committees of the International Union of Pure and Applied Chemistry (IUPAC).

SAFETY OF NOVEL FOODS MEETING

APPENDIX 6

MEMORANDUM ON THE TESTING OF NOVEL FOODS

APPENDIX 6

Department of Health and Social Security
Ministry of Agriculture, Fisheries and Food
Scottish Home and Health Department
Welsh Office
Department of Health and Social Services, Northern Ireland

MEMORANDUM

on

THE TESTING OF NOVEL FOODS

incorporating

GUIDELINES FOR TESTING

by the

ADVISORY COMMITTEE ON IRRADIATED AND NOVEL FOODS

Proof (revised 6.84)

DRAFT

MEMORANDUM ON THE TESTING OF NOVEL FOODS

INTRODUCTION

This Memorandum has been produced for the guidance of companies interested in developing or marketing novel foods, including foods produced by novel processes, in the United Kingdom.

Part 1 provides a definition of novel foods and sets out voluntary, pre-marketing procedures for companies a. to notify the Ministry of Agriculture, Fisheries and Food of new food products which appear to fall within the definition, and b. when appropriate, to seek Ministerial clearance of novel foods.

Part 2 provides Guidelines, which have been elaborated by the Advisory Committee on Irradiated and Novel Foods, on the range of testing which, depending on the degree of novelty and the nature of the product, might have to be carried out satisfactorily before Ministerial clearance of a novel food could be given.

DRAFT

MEMORANDUM ON THE TESTING OF NOVEL FOODS

Contents **Paragraph**

PART 1: THE VOLUNTARY NOTIFICATION AND MINISTERIAL CLEARANCE PROCEDURES

BACKGROUND

Legislative position

1. In England and Wales, the Food and Drugs Act 1955 prescribes offences in respect of the sale of any food intended for human consumption –

a. which, by the addition of any substance as an ingredient in the preparation of the food, the abstraction of any constituent from the food or the subjection of the food to any other process or treatment, has been rendered injurious to health (Section 1);

b. which is not of the nature, substance or quality demanded by the purchaser (Section 2);

c. whose labelling falsely describes the food or is calculated to mislead as to its nature, substance or quality (Section 6);

d. which is unfit for human consumption (Section 8).

2. Ministers have no statutory authority to intervene in the technical development of a food. They may however make Regulations, in respect of food intended for sale for human consumption, to control its composition and the use of any process or treatment in its preparation (Section 4). In this connection, Ministers are empowered to obtain by Order details of the consumption and use of any substance employed in the preparation of the food and details of investigations carried out on the substance (Section 5).

3. Parallel provisions exist in the separate Food and Drugs Acts for Scotland and Northern Ireland.

Development of the procedures

4. As a result of an understanding reached with the Ministry of Agriculture, Fisheries and Food (MAFF) in 1980, the then Food and Drink Industries Council (FDIC) invited its member associations to recommend their member companies to notify MAFF before marketing a novel food so that the nutritional and safety aspects of the food might first be evaluated, in strict confidence, by independent experts. This understanding reflected a recommendation of the Food Standards Committee in its "Report on Novel Protein Foods" (published in 1974).

5. It is now hoped that all companies responsible for developing or marketing novel foods will participate in these arrangements, which have since been modified as described below. The considerable benefits to be derived from improvements in food production are fully recognised and it is emphasised that, in the operation of these voluntary procedures the aim will be to ensure that novelty *per se* is not disadvantaged.

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The Advisory Committee on Irradiated and Novel Foods

6. Since the understanding was reached between MAFF and the FDIC, Ministers have appointed the Advisory Committee on Irradiated and Novel Foods (ACINF): "To advise Health and Agriculture Ministers of Great Britain and the Head of the Department of Health and Social Services for Northern Ireland on any matters relating to the irradiation of food or to the manufacture of novel foods or foods produced from novel processes, having regard where appropriate to the views of relevant expert bodies." These other bodies include the Panel on Novel Foods, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, and the Standing Panel on Hazards from Microbial Contamination of Food. As part of its remit, the ACINF has been given responsibility for evaluating applications for Ministerial clearance of novel foods. The ACINF's membership and secretariat are given in the Appendix.

THE VOLUNTARY PROCEDURES

Definition of a novel food

7. For the purpose of the voluntary procedures, the following definition has been adopted –

> "Novel foods are foods or food ingredients produced from raw material, which has not hitherto been used for human consumption or which has been consumed in only small amounts, or produced by new or ~~severely~~ extensively modified processes not previously used in the production of food".

This definition includes novel foods hitherto consumed in only small amounts in the UK in case those foods might give rise to adverse effects if consumed in larger quantities. As to severe modifications to existing processes, these are, like new processes, potentially capable of bringing about changes of toxicological or nutritional significance. Components extracted from conventional foods by traditional processes are not considered to be novel foods.

Notifications

8. Any company proposing to develop a new food product, or to market an imported one, which appears to fall within the terms of the definition in paragraph 7 above, is requested to notify –

Food Science Division
Ministry of Agriculture, Fisheries and Food
Great Westminster House
Horseferry Road
LONDON SW1P 2AE

All information will be treated in confidence.

9. In practice, it is expected that in most cases the novel features of the

product will not be such as to raise questions of safety to the consumer or of nutritional adequacy. In these cases formal Ministerial clearance would be inappropriate and the company will be advised accordingly. Nevertheless, the company would still need to consider whether the product had been tested adequately to meet the provisions of the Food and Drugs Acts and subordinate legislation.

10. In other cases, the company will be invited to apply for Ministerial clearance of the novel food and will be given an indication of the extent of the data to be submitted and of the likely duration of the evaluation. It will be for the company to decide whether or not to apply.

Evaluation and Ministerial clearance

11. When a formal application is made, it will be referred to the ACINF who, after seeking the views of other expert bodies as appropriate (see paragraph 6 above), will evaluate the information provided and any additional data it might request. The ACINF will advise Ministers of its conclusions but this advice will not be binding on Ministers, who will be responsible for deciding whether or not clearance should be given.

12. The views of Ministers will be notified to the company in writing. It is emphasised that Ministerial clearance of a novel food will not absolve a company from its responsibility to comply with the Food and Drugs Acts and subordinate legislation.

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PART 2: GUIDELINES FOR THE TESTING OF NOVEL FOODS

GENERAL CONSIDERATIONS

Introduction

1. As the tests appropriate to a particular novel food will depend on its source, composition, processing and proposed level in the diet, it will be for the company, in the light of its experience and knowledge of the product, to make the initial assessment of which tests are needed. The tests detailed in the following paragraphs will be necessary for foods of extreme novelty of raw material or process; exceptionally, additional testing may be needed. However, for many novel foods it is expected that some of the suggested tests may, subject to sound scientific justification, be omitted. In all tests, due account should be taken of any further processing to which the novel food will be subjected and of any changes which might occur on storage. It is desirable that, whenever possible, the results of the tests carried out on the novel food should be published in the scientific literature.

Background information

2. It will always be necessary to detail the novel food's nature, its production process and its potential market. This information will be of particular value for assessing the extent of the nutritional and toxicological data required. For example, for some novel foods (such as those unlikely to play a significant role in the diet) extensive nutritional evaluation is unlikely to be required; for others an acceptable presumption of safety may follow from specific chemical studies; and for a few an exhaustive evaluation may be necessary before the product can be considered safe.

3. The source of the novel food or its production process may indicate potential problems. Novel foods produced from plant material may need to be examined for the presence of particular natural toxins or antinutritional factors. Novel fats and oils should be examined for ~~toxic~~ unusual fatty acids (eg erucic acid). Novel foods produced from marine products may need examination for heavy metals and bio-toxins. Production processes involving microorganisms or permitting microbial contamination may indicate the need for examination for toxins or pathogenic microbes. Chemical processing or harsh physical processing may necessitate examination for toxic products or significant damage to nutrients.

4. The structure and size of the market for the novel food and its average intake should be estimated on the basis of its potential use (as indicated by its properties) and the marketing limitations imposed by the projected scale of the production facilities. In addition, groups in the population which might have a much higher intake than the average should be identified and the maximum levels of consumption estimated. As far as possible, any existing foods in the diet expected to be displaced by the novel food should also be identified. This information should enable the test data on the novel food to

be seen in perspective (for example the nutritional aspects of the novel food would have greater significance if it were likely to replace an existing major dietary nutrient source) and any disadvantages in replacing existing foods weighed against any possible advantages.

Specifications

5. An application for clearance of the novel food should include the product specification. This will facilitate assessment of the limits within which the composition of the product is controlled. The specification should be accompanied by details of product variability and of the analytical methods and sampling protocols used to check the specification. If the novel food is so complex that a comprehensive product specification is impracticable (as it might be for a microbial protein), a process specification may be used to augment the product specification.

6. If the application relates to a novel food produced on a pilot scale (which seems likely to be the usual situation), the company will have to demonstrate that when produced in a larger scale plant the food will be nutritionally and toxicologically consistent in all respects with that cleared and that each batch will comply with the pilot scale specification(s). Clearance of a particular novel food will usually be conditional upon its complying with the product and/or process specifications of the material to which the test data relate and possibly upon the inclusion of further criteria within the specification(s).

Chemical composition

7. A thorough understanding of the product and its production process will point to potential problems (see paragraphs 2-4 of Part 2). Therefore, it might be appropriate for suitable chemical analysis of the novel food to precede any biological evaluation. It is not possible to provide a check-list of necessary chemical studies to cover all novel foods but some guidance is given in the following paragraphs; also MAFF might be able to advise. However, chemical analysis need not be regarded as a routine procedure; much will depend on the nature of the product.

8. If the crude protein, total fat or carbohydrate constitutes more than about 10% of the dry matter of the novel food, these components may need to be more fully investigated as follows –

a. Crude protein should be examined for the true protein and non-protein nitrogenous material. Individual amino-acids should be determined as should any unusual or toxic amino-acids if their presence is suspected. (D-amino-acids may be suspected, for example, in bacterial proteins, and lysinoalanine in proteins which have been subjected to alkaline processing conditions). Available lysine measurement is useful in determining whether proteins have undergone severe degradation during processing. Non-protein nitrogenous components such as nucleic acids and amino-glycosides should be determined.

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b. Total fat should be examined for saponifiable and nonsaponifiable components. A full fatty acid spectrum should be determined. Particular attention should be paid to the presence of ~~phospho lipids~~ phospholipids, sterols, cyclic fatty acids and known toxic fatty acids and the amounts of saturated, mono-unsaturated and polyunsaturated fatty acids. This should include an assessment of fatty acids with *trans* double bonds in the monoenoic and polyenoic fractions, *cis, cis,* 9, 12-octadecadienoic acid and fatty acids with chain lengths of 22 and over, both mono- and polyenoic together with peroxidised and degradation products of polyunsaturated fatty acids.

c. Total carbohydrate should be examined for availability. The non-metabolisable fraction ~~(eg fibre, chitin and tannins)~~ (eg fibre and chitin) and also substances such as tannins should be subjected to detailed chemical analysis.

9. After ashing, the novel food should be analysed for the presence of toxic metals (eg lead, arsenic). Depending on its intended use, analysis for metals of nutritional significance (eg iron, zinc, calcium) may be appropriate.

10. The vitamin content of the novel food should be determined if the presence or absence of particular vitamins is likely to be nutritionally significant.

11. If the nature of the novel food or the novel process indicates the possible presence of naturally occurring or adventitious antinutritional factors (phytate, trypsin inhibitors etc) or toxins (haemagglutinins, mycotoxins etc), the product should be analysed for them specifically, by chemical techniques in the first instance. Biological tests, either as part of the nutritional evaluation in the case of enzyme inhibitors or more specifically as part of a mycotoxin screening programme, will provide useful back-up evidence concerning the presence or absence of these contaminants.

NUTRITIONAL STUDIES

Introduction

12. Nutritional studies may be necessary to forecast the likely impact of the novel food on the nutritional status of consumers. If the novel food is expected to play an important dietary role, the results of nutritional studies in animals should be verified in human subjects. The nutritional consequences of the introduction of a novel food into the diet can be judged only in the light of information about its intended uses. As much information as possible should therefore be obtained about potential markets and uses and the likely maximum consumption by particular population groups should be estimated (see paragraph 4 of Part 2). If the novel food would replace a traditional food in the diet, any consequential changes in dietary nutrient intake will need consideration.

13. In 1972 the Committee on Medical Aspects of Food Policy recommended that "any substance promoted as a replacement or alternative to a natural food should be the nutritional equivalent in all but unimportant aspects of the natural food which it would simulate". Accordingly, clearance of a novel food expected to form a significant part of the diet may be conditional upon the food being fortified.

14. Nutrient imbalance and palatability problems during toxicological studies (particularly when high levels of the novel food are incorporated into animal diets) can give rise to misleading conclusions. Nutritional studies will be necessary beforehand to ensure that the test diets will be correctly balanced as regards both macronutrients (eg protein, fat, carbohydrates and metabolisable energy) and micronutrients (eg vitamins and minerals).

Intended use

15. If the novel food is likely to be used as a replacement for or an alternative to a traditional food, it will need to be assessed with particular care. Nutritional data on the novel food should be compared with data on the traditional food. The dietary significance of the traditional food should be established using the National Food Survey or other appropriate means. The influence (beneficial or detrimental) of the introduction of the novel food on the nutrient composition of the diet as a whole should be identified particularly in respect of groups such as children, the elderly and "captive populations" eg hospital patients and schoolchildren.

Effects on nutrients

16. The nutritional value of the novel food should be assessed initially from its chemical composition in respect of both macronutrients and micronutrients, taking into account the effect of any further cooking or processing (including when used as an ingredient in food) and of storage. The possible influence of components in the novel food, such as antinutritional factors (eg inhibitors of enzyme activity or mineral metabolism) on the nutritional value or keeping quality of the remainder of the diet should also be established.

In vivo studies

17. Depending on the nature and intended uses of the novel food, studies in animals may be needed to supplement the chemical studies. If the novel food is likely to form a significant part of the human diet, *in vivo* studies will usually be appropriate to determine its metabolisable energy. If the novel food is intended to be an alternative significant supply of protein, tests on its protein quality as part of the diet will be necessary. *In vivo* studies will also be needed when it is appropriate to determine: a. the availability of vitamins and minerals in the novel food in comparison with the food it would replace; b. the novel foods' components' effect on any added minerals, should any components substantially reduce the mineral availability; and c. any interaction the novel food might have with other items of the diet that would reduce the nutritional value of the diet as a whole.

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18. If the novel food is expected to play an important role in the diet, it may be necessary to verify that the results of animal experiments can be extrapolated to human beings by measuring the availability of nutrients to human subjects.

TOXICOLOGICAL STUDIES

Introduction

19. As explained in the following paragraphs, special considerations apply in the toxicological testing of novel foods, especially when they might constitute a significant proportion of the human diet. In particular –

a. The traditional method of assessing the safety of a food additive, ie allowing a one hundred-fold margin between the maximum amount of the additive likely to be consumed in the human diet and the maximum amount which has no toxic effect when fed to animals, clearly cannot be applied to novel food which would constitute more than one per cent of the human diet. In any case, there are practical limits to the amounts of certain foods which can be added to animal diets without adversely affecting the animals' nutritional status and health.

b. A novel food, once it has been adequately tested in appropriate animal and *in vitro* systems, should always undergo tolerance testing, including monitoring for possible allergenicity in small groups of normal human volunteers under controlled conditions and under medical supervision.

Studies in laboratory animals

20. The general principles of animal husbandry to be adopted when assessing the safety of novel foods are set out in the DHSS "Guidelines for the Testing of Chemicals for Toxicity" (HMSO, 1982). This document should also be consulted for further details of the tests discussed in paragraphs 25-35. Reference should also be made to the documents mentioned below for certain details of specific tests.

21. Before starting animal studies, it is desirable to investigate the palatability of the test diet in the test animals. If a palatability problem is encountered, it may be necessary to increase the amount of novel food to the required level gradually. Paired-feeding techniques should be used if the problem cannot be overcome.

Metabolic ~~studies~~ fate

22. As foods are usually complex mixtures of chemicals, studies on the metabolic fate of every constituent of the novel food would be impracticable. However, if it is suspected that contaminants or minor components are the cause of toxicity, the metabolism of the suspect chemicals should be

investigated. Also, if the novel food, or a major component of it, consists of a new chemical compound which does not normally occur in the diet (eg a novel carbohydrate), studies of the metabolic fate of the new compound will be appropriate.

23. Changes in normal excretory functions caused by the novel food will be relevant, and analysis of urine and faeces may give important information. For example, a novel food may alter the gut flora drastically, or may encourage preferential loss of a mineral or vitamin to the detriment of the good health of the study animals.

Acute toxicity studies

24. As a novel food will usually consist mostly of compounds unlikely to produce acute toxic effects (carbohydrates, lipids and proteins), acute toxicity studies will normally be inappropriate.

Sub-acute toxicity tests

25. The novel food's safety should be confirmed by sub-acute toxicity studies preferably in a rodent species and in one or more other species. The studies should be started shortly after the animals are weaned and extend throughout the period of rapid growth (a study period of 90 days will be the normal duration but this may have to be varied, eg a longer period may be necessary in the case of longer-lived species.)

26. The novel food should be fed to different groups of animals at a minimum of two levels of incorporation into the diet. A control group, which is not fed any of the novel food, must be included in the study. The higher level of incorporation should be set according to the likely level of the novel food in the human diet. In all cases this level must be substantially in excess of the anticipated exposure level in man, which in some cases will imply feeding at the maximum practicable level. The second level of incorporation should be set appropriately between the high-dose level and the nil-dose level of the control groups. The inclusion of a minimum of two separate groups of animals receiving the novel foods at different levels in the diet will facilitate distinction between dose-related (toxic) and non-specific effects. Numerous dietary incorporation levels in animal experiments will not generally be of use since the establishment of a precise no-adverse-effect level is inappropriate in the safety assessment of a novel food on account of the impracticability of achieving an adequate safety margin between the no-effect level in animals and the expected consumption of the novel food in humans (see paragraph 19a).

27. It must be ensured that the novel food does not distort the nutritional status of the test animal and that the test and control diets have the same nutritive value in terms of both macronutrients and micronutrients. Comprehensive nutritional analyses of the novel food and of the standard laboratory diet should therefore be obtained to determine any need, in the preparation of the diet for the test group of animals, to supplement or reduce any of the standard diet components when mixing in the novel food.

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general

28. Animals should be observed regularly for effects on behaviour and state of health. At reasonable intervals throughout the study, data should be obtained from all the animals on body weight, food and water consumption. In addition, it will generally be useful to obtain data on haematological parameters, ophthalmic examination, blood chemistry, urinalysis and faeces analysis and it may sometimes be necessary to measure hormone levels and mineral and vitamin excretory levels. Any test thought appropriate to the biological properties of the novel food should also be included. Data on organ weights, gross pathology and histopathology should be obtained from all the animals at the end of the study period. Histological studies should be performed in the control and high-dose groups of animals and, if any histological abnormalities are found, in the intermediate-dose group also. As it is not possible to have a large safety factor between the quantity ingested by the test animal and the likely human exposure, it is particularly important that the parameters for assessing the novel food are chosen carefully to include monitoring of all its possible toxic effects.

Long-term toxicity and carcinogenicity tests

29. The need for long-term animal feeding studies will depend on the nature of the novel food. In many cases a long-term study could be omitted provided the results of sub-acute toxicity and mutagenicity studies were satisfactory, but in others, especially if the raw material or the process is particularly novel, a long-term study will be needed. When a long-term study is indicated, a sub-acute study will not necessarily be required, but it might be advisable to conduct one to facilitate the planning of the long-term study.

30. Long-term studies should be performed over a major part of the test animals' lifetime so that normally 50-80% of animals will have died from causes other than toxicity attributable to the test material when the experiment is terminated. This would be about two years in rats and about 18 months in mice and hamsters which are the most commonly used species in long-term studies. Strains of animals which do not have a high background incidence of spontaneous tumours, but which are reasonably susceptible to known carcinogens, should be chosen in order to optimise the study for the detection of any carcinogenic effects of the novel food. More details about carcinogenicity studies are given in the DHSS "Guidelines for the Testing of Chemicals for Carcinogenicity" (HMSO, 1982).

or highly variable

31. Main groups should consist of not less than 50 animals of each sex. It is advisable that control groups should be larger than test groups. Historical data for animals of the same strain, although often useful, should not be regarded as a substitute for adequate numbers in the control groups.

32. Appropriate levels of dietary incorporation of the novel food should be used, following the same considerations as apply to a short-term study (see paragraphs 25-28). It is particularly important to avoid distortion of the nutritional status of the test animals.

Embryotoxicity (including teratogenicity) and reproduction studies

33. The general principles and techniques of embryotoxicity and reproduction studies are described in numerous publications, for example, Appendix 6 of the DHSS "Guidelines for the Testing of Chemicals for Toxicity" (HMSO, 1982). The highest practicable dose of novel food (as defined in paragraph 26) should be included, though any distortion of the nutritional status of the test animals must be avoided.

34. If the novel food is produced by an extremely novel process and/or from an extremely novel source, a multigeneration study may be necessary. A two generation, two litter design would normally be suitable. The physical features and behaviour of the offspring should be investigated, and oogenesis and spermatogenesis should be monitored.

Mutagenicity studies

35. Mutagenicity tests are designed to measure genetic damage. While not all mutagens are carcinogens and some carcinogens are not mutagens, there is a broad correlation between the ability to induce genetic damage and the ability to induce cancer. Also, mutagenic damage to the germ cells may itself present a hazard to future generations. There are problems in the interpretation of mutagenicity tests on novel foods, particularly in view of the presence of numerous mutagens in naturally-occurring foodstuffs. Nevertheless, such studies may be valuable and, whether or not long-term toxicity studies are undertaken, mutagenicity studies will be needed on the novel food. The DHSS "Guidelines for the Testing of Chemicals for Mutagenicity" (HMSO, 1981) details the 'basic package' of recommended tests. *In vitro* mutagenicity testing of the novel food may present particular problems owing to the presence in the growth medium of nutrients from the food, making it necessary to use special bacterial strains or suitable extraction procedures prior to testing.

STUDIES IN HUMANS

Introduction

36. Before embarking on human studies (other than for evaluation of palatability), the novel food must have been shown to cause no adverse effects in animals. The necessary amount of testing in animals will depend on the degree of novelty of the food, but for extremely novel products a minimum of sub-acute feeding studies in two species and relevant mutagenicity tests will be essential.

37. It would be acceptable for the novel food to be tested in human volunteers without any studies on its effects on reproduction and its embryotoxicity, provided women of child-bearing age were excluded and provided it had otherwise been adequately tested. However, tests in animals for effects on reproduction (see paragraphs 34-35) will normally be necessary before the food is used in a large-scale acceptability and marketing trial.

The selection of variables to be measured may be influenced by the results of the animal studies.

38. All studies carried out on human subjects must take into account not only scientific objectives but also ethical and legal considerations (see Appendix 10 of the "Guidelines for the Testing of Chemicals for Toxicity" (HMSO, 1982). This is particularly important when testing a novel food, because there is no direct benefit to the participants.

39. In order to minimise the number of volunteers exposed to the novel food in the early stages of its development, all possible information should be obtained from each study. Throughout each experiment regular urinalyses, faecal analyses, clinical chemical and haematological investigations, and renal and hepatic function tests should be performed, together with any relevant specialised tests which allow the monitoring of specific organ function.

Preliminary single-dose studies

40. It is generally advisable that the initial human study should involve the feeding of a single meal containing the novel food at a known dose level to one volunteer at a time. Time should be allowed for any reactions to show before the test dose is given to another volunteer. The results of the study might make it possible to predict likely effects in man and to choose more suitable animal models for long-term studies.

Further studies in humans

41. When the novel food has been shown to have no harmful effects when fed in single doses, a study involving the feeding of the novel food for a short period (initially about four weeks with follow-up studies of longer duration) should be performed. Different diets, incorporating different levels of the novel food, should be fed to different groups of volunteers. The dietary levels should be related to the anticipated levels of human exposure. Each group should consist of a sufficient number of volunteers to allow reliable statistical analysis of the results. All studies should include concurrent control groups receiving a diet not incorporating the novel food. Those in the control groups should be of similar age, size, sex, alcohol intake and smoking habits to those in the test groups. Each test group and its paired control group should be fed diets which are as similar as possible.

42. In addition to having control groups, it may be useful to organise studies in which the test groups are fed diets incorporating and not incorporating the novel food in sequential periods, so that each volunteer acts as his own control: blind crossover trials are the most satisfactory.

43. Retrospective analysis should be avoided; instead, a careful note should be made at the time of all reactions experienced by controls and volunteers in case they are later seen to have a relevance to the novel food. Particular attention should be paid to gastrointestinal symptoms and possible allergic reactions, so that an assessment of the digestibility of the novel food and of the volunteer's tolerance to it can be made.

44. If the novel food is intended for use by a certain community or

section of the community (eg in a particular country, or by diabetics), at least one study should be conducted in the group of people for whom the food is intended.

45. If the novel food has been tolerated well by the volunteers at fixed dietary levels, it may be useful to feed it *ad libitum* for a short period of time in order to assess its acceptability.

Health monitoring of employees

46. The health of workers coming into contact with the novel food (eg laboratory staff, workers in the manufacturing plant) should be monitored, not only because they are dealing with an incompletely tested (and therefore potentially harmful) substance, but also because adverse effects (eg allergenicity) of the novel food may be revealed. The state of the food when exposure occurs will be significant; for example, the risk of inhalation of food particles while handling the raw material may present an increased risk of allergic and other reactions developing. Thus, it should be noted whether laboratory and manufacturing staff are exposed to the novel food in its raw or cooked state, or both.

Allergenicity studies

47. It may be necessary to conduct allergenicity studies on the novel food because of its composition (eg highly proteinaceous) or because the results of animal or human feeding studies suggest that the food might produce allergic effects in some people. However, one way of detecting any induced allergenicity in the exposed population during pre-marketing trials is to arrange for a particular standardised antigen made from a component of the novel food to be included in the screening profile used in a local allergy clinic. This is usually best organised through a specialised allergy testing unit. In order to detect possible induced allergenicity of the novel food in the general population, it will generally be essential to monitor a large number of people. Where allergenicity is suspected, types of immunological tests required to clarify the situation should be established in consultation with relevant specialists.

Large-scale acceptability and marketing trials

48. When the novel food's safety has been demonstrated by the studies indicated above, large-scale acceptability and marketing trials should be undertaken.

49. For products of extreme novelty, the local medical services responsible for the areas where the novel food is to be tested should be alerted so that they may take it into account when evaluating any unusual disease patterns that may appear during or after the test period. As large numbers of people will be involved in the trials, it may be possible to obtain information about any rare adverse reactions to the novel food (eg allergic reactions) which might not have been observed in earlier human studies. The

extent to which health monitoring should be performed will depend on the nature of the novel food and the results of previous toxicological investigations, but in any case it will probably be advisable to arrange for basic medical surveillance of the individuals involved. To ensure that all information relevant to safety assessment in a large-scale trial is obtained, one medical practitioner should be given overall responsibility for the health monitoring, and it may also be useful to restrict the trial to a defined geographical area.

Special groups

50. In addition to establishing the safety of the novel food in the general population special attention needs to be paid to vulnerable groups such as pregnant women, children, the elderly, diabetics and any other group thought to be especially at risk, eg subjects with an inborn error of metabolism. (The severity of the risk will dictate the protective measures necessary, eg appropriate labelling might be adequate.)

Interaction with drugs

51. Particular care should be taken to establish whether the novel food might interfere with the absorption or action of commonly-used drugs. If there appears to be such a possibility, appropriate pharmacological studies should be performed.

Progress in the future

52. As new methods of investigation become available in special areas, eg allergy testing, they should be incorporated into the above test procedures as and where appropriate.

REFERENCES

DHSS "Guidelines for the Testing of Chemicals for Toxicity" (HMSO, 1982)

DHSS "Guidelines for the Testing of Chemicals for Mutagenicity" (HMSO, 1981)

DHSS "Guidelines for the Testing of Chemicals for Carcinogenicity" (HMSO, 1982)

APPENDIX

THE ADVISORY COMMITTEE ON IRRADIATED AND NOVEL FOODS

CHAIRMAN

Sir Arnold Burgen MD FRCP FRS
Master of Darwin College, University of Cambridge

MEMBERS

Professor J W Bridges BSc PhD FRSC CChem FIBiol MRCPath MInstEnvSci
Director of the Robens Institute of Industrial and Environmental Health and Safety, University of Surrey

Professor J D Bu'Lock BA PhD
Head of Microbial Chemistry Laboratories, University of Manchester

Dr J C Gould BSc MD FRCPE FRCPath FRSE
Director of the Central Microbiological Laboratories, Edinburgh

Professor J Hawthorn BSc PhD ARCST CChem FRSC FIBiol FIFST FRSE
Professor of Food Science, University of Strathclyde

Professor W P T James MA MD FRCP
Director of the Rowett Research Institute, Aberdeen

Dr B E B Moseley BSc PhD
Senior Lecturer in Microbiology, University of Edinburgh

Dr A N B Stott MB ChB FFOM
Chief Medical Officer, United Kingdom Atomic Energy Authority

Dr A J Swallow PhD DSc ScD FRSC
Head of the Biophysical Chemistry Division, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester

Professor E D Wills MA MSc PhD ScD
Head of the Department of Biochemistry, St Bartholomew's Hospital Medical College, London

EX OFFICIO MEMBERS

Professor A E Bender BSc PhD FRSH FIFST
Former Professor of Nutrition and Dietetics, University of London
(Chairman of the Panel on Novel Foods)

Professor P Turner MD BSc FRCP
Professor of Clinical Pharmacology, St Bartholomew's Hospital Medical College, London
(Chairman of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment)

Dr J E M Whitehead MA MB FRCPath DipBact
Director of the Public Health Laboratory Service, London
(Member of the Standing Panel on Hazards from Microbial Contamination of Food)

ADVISER ON THE INDUSTRIAL APPLICATION OF FOOD IRRADIATION

F J Ley BSc FRSH FIBiol
Director of Irradiated Products Ltd, Swindon

JOINT SECRETARIES

Medical: Dr J H Steedman, Department of Health and Social Security
Scientific: Dr D A Jonas, Ministry of Agriculture, Fisheries and Food
Administrative: B H Wiggins, Department of Health and Social Security

SAFETY OF NOVEL FOODS

APPENDIX 7

THE COUNCIL OF THE EUROPEAN COMMUNITIES
COUNCIL DIRECTIVE OF 18 APRIL 1983
ON THE FIXING OF GUIDELINES FOR THE ASSESSMENT OF CERTAIN PRODUCTS USED IN ANIMAL NUTRITION

COUNCIL DIRECTIVE
of 18 April 1983
on the fixing of guidelines for the assessment of certain products used in animal nutrition

(83/228/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 82/471/EEC of 30 June 1982 concerning certain products used in animal nutrition (1), and in particular Article 7 thereof,

Having regard to the proposal from the Commission,

Whereas Directive 82/471/EEC provides that the products belonging to certain groups must be examined on the basis of a dossier forwarded officially to the Member States and the Commission;

Whereas such dossiers must make it possible to verify that the products in question comply with the general principles laid down in the Directive in respect of the inclusion of new products in the Annex;

Whereas it has been found necessary to provide for the dossiers to be compiled in accordance with common guidelines defining, for each principle, the scientific data which make it possible to identify and characterize the products concerned and the studies necessary in order to evaluate their nutritional properties and biological effects; whereas these guidelines must be applicable on the date on which Directive 82/471/EEC itself enters into force;

Whereas the guidelines are intended primarily as a general guide; whereas, depending on the nature of the product or its conditions of use, the extent of the studies necessary in order to evaluate its properties or its effects may vary;

Whereas the guidelines have been drawn up on the basis of present scientific and technical knowledge and they may be adapted if necessary to any developments in this sphere,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Member States shall prescribe that the dossiers on the products listed in points 1.1 and 1.2 of the Annex to Directive 82/471/EEC are to be compiled in accordance with the guidelines set out in the Annex hereto.

Article 2

Member States shall bring into force the laws, regulations or administrative provisions necessary in order to comply with this Directive by 13 July 1984 at the latest. They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Luxembourg, 18 April 1983.

For the Council

The President

I. KIECHLE

(1) OJ No L 213, 21. 7. 1982, p. 8.

ANNEX

GUIDELINES FOR THE ASSESSMENT OF CERTAIN PRODUCTS USED IN ANIMAL NUTRITION

General aspects

These 'guidelines' constitute a guide intended to establish dossiers on products listed in items 1.1 and 1.2 of the Annex to Directive 82/471/EEC, which have been obtained from culturing micro-organisms and which are likely to be admitted as a new source of proteins in animal nutrition. These dossiers should enable an assessment of such products based on the present state of knowledge and should ensure their compliance with the fundamental principles laid down for permitting their use, which are the subject of Article 6 (2) of the abovementioned Directive.

All the studies outlined in this document may be required and, if necessary, additional information may be requested. As a general rule, all the information necessary to establish the identity of the micro-organism and the composition of the culture medium, and also the manufacturing process, characteristics, presentation, conditions of use, methods of determination and nutritional properties of the product must be provided. The same applies to the information necessary to assess the tolerance of the product by the target species and the risks for man and the environment, which could result directly or indirectly from the use of the product. The toxicological studies required for this purpose will depend on the nature of the product, the animal species concerned and the metabolism of the product in laboratory animals.

The documentation to be provided should include detailed reports, presented in the order and with the numbering proposed in these guidelines and should be accompanied by a summary. The omission of any proposed studies should be justified. The publications quoted as references should be attached.

Observations

The term 'product', as used in these guidelines, refers to any proteinaceous product in the state in which it will be presented as feedingstuff or component of a feedingstuff.

Any modification in the manufacturing process or in the conditions of use of a product will require notification and, if necessary, additional documentation for a new assessment.

Presentation of studies

I. Micro-organism, culture medium and manufacturing process, characteristics of product, presentation and conditions of use, methods of determination

II. Studies on the nutritional properties of the product

III. Studies on the biological consequences of the use of the product in animal nutrition

IV. Other relevant studies

SECTION I

MICRO-ORGANISM CULTURE MEDIUM AND MANUFACTURING PROCESS, CHARACTERISTICS OF PRODUCT, PRESENTATION AND CONDITIONS OF USE, METHODS OF DETERMINATION

1. MICRO-ORGANISM

1.1. Classification, provenance, morphology, biological properties, any genetic manipulation.

1.2. Innocuity, possible survival outside the fermenter and any environmental consequences.

1.3. Constancy and purity of strains cultivated. Methods used to check these criteria.

2. CULTURE MEDIUM AND MANUFACTURING PROCESS

2.1. Composition of substrate, added substances, etc.

2.2. Manufacturing, dessication and purification processes. Devitalizing process for micro-organisms. Methods used to check the constancy of composition of the culture product and the detection of any chemical, physical and biological contamination during production.

2.3. Technical processes of preparation for use.

3. CHARACTERISTICS OF PRODUCT

3.1. Physical and physico-chemical properties: macro- and micro-morphology, particle size, density, specific weight, hygroscopicity, solubility, electrostatic properties, etc.

3.2. Chemical composition and characteristics.

3.2.1. Content of moisture, crude protein, crude fat, crude cellulose, crude ash, carbohydrates. Limits of variation of these contents.

3.2.2. Content of total ammonium, amide, nitrate and nitrite nitrogen, nucleic acids, protein. Qualitative and quantitative composition of total and free amino acids, and purine and pyrimidine bases.

3.2.3. Qualitative and quantitative composition of total lipids: fatty acids, non-saponifiable matter, lipid soluble pigments, phospholipids.

3.2.4. Composition of the carbohydrate fraction.

3.2.5. Qualitative and quantitative composition of inorganic components.

3.2.6. Qualitative and quantitative composition of vitamins.

3.2.7. Qualitative and quantitative composition of the other constituents: additives, residues of substrate and solvents, other potentially harmful residues of the metabolism of the substrate, of the culture medium, of the manufacturing process.

3.3. Microbiological contamination of the product.

3.4. Behaviour and stability of the product, as such and when mixed with feedingstuffs in current use, during storage.

4. PRESENTATION AND CONDITIONS OF USE

4.1. Proposed names of marketing the product.

4.2. Proposed formulations for marketing the product.

4.3. Intended use of the product in animal nutrition. Intended concentrations in the complete feedingstuffs and in the intended quantities in the daily rations for the animal species concerned.

5. METHODS OF DETERMINATION

Qualitative and quantitative methods for determination of the product in complete and complementary feedingstuffs.

NB: Description of these methods should be accompanied by information as to specificity, sensitivity, limits of detection, margin of error, possible interferences by other substances. Samples of the product in its various proposed presentations should be available.

SECTION II

STUDIES ON THE NUTRITIONAL PROPERTIES OF THE PRODUCT

1. ASSESSMENT OF PROTEIN VALUE

1.1. Chemical, biochemical and microbiological studies.

1.2. Studies on laboratory animals, compared with reference proteins.

2. STUDIES ON TARGET SPECIES

The following studies should be performed on each target species in comparison with a control group receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen, for ruminants of total nitrogen.

2.1. Protein and energy supplementation value of the product in the rations under the proposed conditions of use at various physiological stages of the animals (e.g. growing period, pregnancy, laying).

2.2. Influence of the product under the proposed conditions of use on growth rate, feed conversion rate, morbidity, mortality.

2.3. Optimum nutritional levels of incorporation of the product in the rations.

2.4. Effect of the product under the proposed conditions of use on the technological, organoleptic or other qualities of edible products of animal origin.

3. EXPERIMENTAL CONDITIONS IN THE STUDIES ON TARGET SPECIES

Give a detailed description of the tests performed and provide the following data:

3.1. Species, breed, age and sex of the animals, identification procedure.

3.2. Number of test and control groups; number of animals in each group (the number should be large enough for statistical analysis using suitable statistical parameters).

3.3. Levels of incorporation of the product, qualitative and quantitative composition of the ration and its analysis.

3.4. Location of each experiment, physiological state and animal health conditions, rearing conditions (these should reflect those used in practice in the Community).

3.5. Exact duration of testing and date of the analyses performed.

3.6. Adverse effects which occurred during the experiment and time of their appearance.

SECTION III

STUDIES CONCERNING THE BIOLOGICAL CONSEQUENCES OF THE USE OF THE PRODUCT IN ANIMAL NUTRITION

The studies outlined in this section are intended to permit assessment of the safety in use of the product in the target species, and of the risks for man and the environment which could result directly or indirectly from this use. The toxicological studies required for this purpose will depend on the nature of the product, the animal species concerned and the metabolism of the product in laboratory animals.

1. STUDIES ON TARGET SPECIES

The following studies should be performed on each target species in comparison with a control group receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen, for ruminants of total nitrogen.

1.1. Maximum incorporation rates of the product in the daily ration without producing any adverse effect.

1.2. Possible effect of the product on fertility and reproduction, if appropriate.

1.3. Effects of ingestion of the product under the proposed conditions of use on micro-organisms of the flora of the alimentary tract and on colonization of pathogens in the alimentary tract.

1.4. Investigation under the proposed conditions of use of possible residues of the product (substrate, culture medium, solvents, contaminants) in edible products of animal origin.

1.5. Investigation under the proposed conditions of use of possible residues of the product (substrate, culture medium, solvents, contaminants) in excreta.

2. STUDIES ON LABORATORY ANIMALS

2.1. **Metabolism**

Fate of the product in the animal: absorption, accumulation, biotransformation, elimination.

2.2. **Mutagenicity**

Investigations of potential mutagenicity due to contaminants (in particular mycotoxins and bacteria) or residues of the product (substrate, culture medium, solvents) including *in vitro* screening tests using metabolic activation systems.

2.3. **Toxicological studies**

The following studies should be performed in comparison with control groups receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen. Toxic effects should be investigated to elucidate their cause and mechanisms and to ascertain that they do not result from nutritional imbalance or from an overdosage of the product in the diet.

2.3.1. *Subchronic toxicity (at least 90 days)*

In general, these studies should be carried out on two animal species, one of which being a rodent. The product should be administered in the daily ration in at least two levels of incorporation. These should be chosen so as to determine, if possible, a no-effect level and a level showing some adverse effect. The animal groups should contain an adequate number of subjects of each sex. A control group should always be included.

All relevant biological data should be recorded at appropriate intervals, particularly data on growth rate, feed consumption, haematology, urine analysis, biochemical parameters, mortality, organ

weights, gross pathology and histopathology of major organs and tissues. The results should be presented in detail and, as far as possible, should include statistical assessment.

2.3.2. *Chronic toxicity*

In general, chronic toxicity studies should be carried out on two animal species, one of which being a rodent. The product should be admininstered in the daily ration in at least two levels of incorporation. Experiments should extend for a minimum of two years in the rat or 80 weeks in mice. The animal groups should contain an adequate number of subjects of each sex. A control group should always be included.

The biological examinations mentioned under point 2.3.1 should be carried out preferably on a small satellite group of animals (a group separated from and dependent upon the main group) at appropriate intervals throughout the experiment and on the surviving animals at the end of the experiment.

2.3.3. *Carcinogenicity*

For assessing carcinogenicity, particular attention should be paid to the time of appearance, the histological types of any observed tumours and their incidence. Any effect on the incidence of tumours and/or the incidence or progress of diseases should be assessed by reference to control groups, as indicated in paragraph 2.3. The results should be presented in detail and, as far as possible, should include statistical assessment.

2.4. **Other studies**

Reproduction studies should extend over at least two filial generations and may be combined with embryotoxicity including teratogenicity studies. Particular attention should be paid to fertility, fecundity and observation on post-natal development of litters. Any other method that is scientifically justifiable and likely to produce measurable results (e.g. relay toxicity) may be provided.

2.5. **Experimental conditions in the studies on laboratory animals**

Give detailed description of the tests performed and provide the following data:

2.5.1. Species, breed, strain and sex of animals.

2.5.2. Number of test and control groups, number of animals in each group (the number should be large enough for statistical analysis using suitable statistical parameters).

2.5.3. Levels of incorporation of the product, qualitative and quantitative composition of the ration and its analysis.

2.5.4. General rearing conditions throughout the period of testing.

2.5.5. Exact duration of testing and date of examinations performed.

2.5.6. Rate and timing of deaths for the various test groups.

2.5.7. Clinical symptoms and pathological alterations which occurred during the experiment and time of their appearance.

3. STUDIES CONCERNING THE ENVIRONMENT

Depending on the nature of possible residues of the product (substrate, culture medium, solvents, contaminants) in excreta of target species, data on the fate of these residues in manure, soil and water and also their effects on soil biology, plant growth and aquatic life may be required.

SECTION IV

OTHER RELEVANT STUDIES

Depending on the nature and the conditions of use of the product, data on allergic effects, on irritation of the skin and mucus membranes of the eye, respiratory or digestive tract may be required to assess possible risks in handling the product and to prevent them.

SAFETY OF NOVEL FOODS MEETING

APPENDIX 8

NOVEL FOODS - ESTIMATED TESTING COSTS

APPENDIX 8

NOVEL FOODS - ESTIMATED TESTING COSTS

I. Introduction

The testing cost estimates discussed here for novel foods are based on the assumption that individual products will require different levels of toxicity testing depending on their degree of novelty, the novelty of the raw materials used in their production and the processes involved. Therefore, to give maximum flexibility combined with the ability to recognize unacceptable toxicity as inexpensively as possible, the costing of the toxicity program is arranged sequentially as a series of programs of increasing cost and increasing duration. The level to which it is necessary to undertake toxicity testing of an individual novel food will be a difficult decision using the advice of highly skilled nutritionists, analysts and toxicologists in combination with those responsible for product regulation.

It is visualized that developers of novel foods may wish to take the product to one of the many reputable commercial establishments for toxicity testing. Approximate testing costs are given in Canadian dollars and it should be realized that these will vary between one testing house and another, with the standards to be met as well as with the amount of testing that is required. For example, if Good Laboratory Practice standards are required this will inevitably raise costs by 25-30% over those estimated here.

II. Program

a) Microbial Program *

This program represents a minimum to establish freedom of the novel food from microbial contamination that might lead to human disease.

Total bacteria, Escherichia coli, salmonella, clostridium perfringes and coagulase-positive staphylococus. 2,000.00

b) Chemical Program *

The tests outlined here are a minimum to ensure that the product is not chemically contaminated. Knowledge of the product may suggest other specific analyses based on the degree of contaminants during processing or storage.

(i) Tests for solvent residues (such as hexane and other organic solvents, inorganic acids and alkalis) and known toxic factors such as glucosinolates, gossypol, aflatoxins, lysinoalanine, heavy metal contamination. 1,000.00

(ii) Tests required to define substrates in the case of SCP products (such as chain length and purity, presence of any potentially carcinogenic polycyclic aromatic hydrocarbons, etc. 2,000.00

c) Nutritional Program

The purpose of this program is to ensure that the novel food is nutritionally acceptable and does not contain components such as novel or unusual fatty acids whose toxicity is not adequately known.

Proximate composition, amino acid profile, protein efficiency ratio, mineral analyses including heavy metals, vitamin analyses including 15 vitamins, fatty acid composition, reducing sugars. 6,000.00 (Total)

* The costs of microbial and chemical testing listed above represent absolute minimum costs for basic testing. Actual costs may range considerably higher depending on the complexity and length of tests deemed appropriate.

d) Toxicity Program

The toxicity program is performed in experimental animals and is an essential precursor of the clinical tests described in the next section. A very limited program is suggested including a subchronic study, a reproduction study in one or three generations, a genotoxicity test (Ames) and a chronic study designed to determine degenerative changes as well as cancer. This does not in any way attempt to cover the possible complete range of tests which might also include tests for behavioral deficits or immunotoxicity, for example.

Subacute study (one species)	40,000.00
Reproduction study (one species, one generation)	40,000.00
Reproduction studies (one species, three generations)	120,000.00
Short-term tests for carcinogenicity (Ames Test)	1,200.00
Chronic toxicity/carcinogenictiy (rat and mouse)	1,000,000.00

e) Clinical Program in Humans

The clinical program is designed to ensure that the novel food that appears acceptable from animal based toxicity tests (above) does not produce unexpected adverse effects in humans. this program should be based in a hospital or clinic and will involve close clinical monitoring of the volunteer population exposed to the novel food or placebo.

Determination of product acceptability and physiological tolerance (involving 50 subjects, four product intake levels for a period of 60 days).	70,000.00
Determination of nitrogen balance (involving 8 subjects fed four protein levels for a period of 11 days).	15,000.00
Measurement of various changes, e.g. in amounts of albumin, amino acids, uric acid and enzyme activity in blood plasma or serum.	2,000.00

f) Initial Marketing Surveillance Program

The need may be perceived for a form of population monitoring for unexpected adverse effects when the novel food is first introduced to the market place. The cost will depend on the complexity of the monitoring program.

III. Testing Packages

The level of testing required for a novel food will ultimately depend on the degree of novelty, the expected overall consumption rate by the population, and expected consumption by high level users (outliers). The following gives the estimated costs in Canadian dollars and the duration of a number of testing packages.

Package 1: Microbial and Chemical Tests. Time: 1-2 months
Minimum: $5,000.00

A package of this sort might be expected to be done batchwise if a large scale production of a novel food is envisioned. Novel foods with known or predictable toxic principles would require appropriate specific tests (e.g. novel potatoes for solanine).

Package 2: Microbial, Chemical and Nutritional Tests. Time: 3-4 months
Minimum: approximately $11,000.00

Again, this package might form one basis for defining batchwise product acceptability in cases such as single cell protein.

Package 3: Microbial, Chemical, Nutritional and (minimum) Toxicological Tests. Time: 14 months Minimum: $92,000.00

Minimum toxicology testing would consist of subacute study (one species), reproduction study (one generation) and genotoxicity test (one).

Package 4: Microbial, Chemical, Nutritional and (full) Toxicological (including carcinogenicity) Tests. Time: 60 months Minimum: $1,172,000.00

A full toxicological package will consist of subacute study, genotoxicity test (one), three generation reproduction study, cancer and chronic study.

Package 5: Microbial, Chemical, Nutritional, (minimum) Toxicological and Clinical Tests. Time: 24 months Minimum: $179,000.00

Package 6: Full Test: Package 4 and Clinical Tests. Time: 70 months Minimum: $1,259,000.00

VI. Conclusion

The size and scope of the testing data package required for a specific novel food raises important and difficult questions at the technical, policy and regulatory levels. The requirements set out in this text are minimal and should not necessarily be construed to encompass all that may be needed in specific circumstances.

EDITOR'S NOTE:

Dr. I.C. Munro of the Canadian Centre for Toxicology at the University of Guelph suggested that a minimum novel foods testing facility might include 15 to 20 scientists with an annual operating budget of roughly $5 to $8 million in Canada. Capital costs for establishing such a laboratory might similarly be in the order of $5 to $8 million.

Dr. Munro strongly recommends contract testing of novel food products especially in countries where the necessary expertise is in short supply.